



# Echo<sup>®</sup> Dose-Response Software

**Destination Plate Designer**

Step 2: Define the dose-response data points  
Select the number of data points and the number of controls per dose-response curve

Please fill out the table below in its entirety.

#	Source Concentration	Transfer Volume (μL)	Backfill Volume (μL)	Assay Volume (μL)	Final Concentration
1	0.01 M	25.0	50	10	2.488E-05 M
2	0.01 M	7.5	67.5	10	7.453E-06 M
3	0.01 M	2.5	72.5	10	2.482E-06 M
4	0.0001 M	75.0	0	10	7.500E-07 M
5	0.0001 M	25.0	50	10	2.488E-07 M
6	0.0001 M	7.5	67.5	10	7.450E-08 M
7	0.0001 M	2.5	72.5	10	2.482E-08 M
8	1E-06 M	75.0	0	10	7.500E-09 M
9	1E-06 M	25.0	50	10	2.488E-09 M
10	1E-06 M	7.5	67.5	10	7.450E-10 M
11	1E-06 M	2.5	72.5	10	2.482E-10 M
12	Positive Control	75.0	0	10	0.000E+00 M

Dose-Response Curve  
Number of data + control points per curve: 13  
Include Controls in Dropdown Lists   
Assay Volume: 10.00 μL  
Automatic DMSO Backfill   
Perform Backfill:  First  Last

Replicates  
Number of Replicates: 1  
 Same Plate  
 Different Plates

Curve Fill Direction:  Compound Fill Direction

**Destination Plate Designer**

Step 3: Choose a name for the destination plate template  
The name of the template must be unique within the system.

Template Name: 1530-well destination plate

Plate Type: Greiner\_153048basePS\_782096

Plate Preview:

Legend:  
 Compound Conc.  
 Control  
 DMSO  
 Reserved  
 Available

**Source Plate Designer**

Step 1: Design the source plate template  
Select the wells in the microplate and then click on one of the buttons in the Legend to define the type for the selected wells in the source plate template. When finished, click on Next.

Source Plate Type: labcye\_24x96\_1671

Well Selection Actions:  
 Compound Conc.  
 Control  
 DMSO  
 Reserved  
 Available

Replicate Pattern:  
Click on the button below after you have selected the wells to copy as compounds.  
 Replicate Selected Pattern  
 Copy Replicate Pattern

Direction:  
 Across  
 Down

## User Manual Software version 1.2

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Echo Dose-Response User Manual  
Labcyte Part Number 001-5722 Revision 2  
December 2007

The information contained in this manual is subject to change without notice.

Any unauthorized changes or modifications to the Echo® Dose-Response application will void its warranty.  
Contact Labcyte, Inc. prior to making any change or modification.

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For research purposes only; not for use in diagnostics.

The Echo liquid handler is covered by one or more of the following patents:

6,416,164; 6,548,308; 6,603,118; 6,612,686; 6,642,061; 6,666,541; 6,707,038; 6,710,335; 6,746,104; 6,802,593; 6,808,934;  
6,809,315; 6,849,423; 6,855,925; 6,869,551; 6,893,836; 6,893,115; 6,916,083; 6,932,097; 6,938,987; 6,938,995; 6,991,917;  
7,070,260; 7,090,333; European Patent EP 1337325.

Additional patents pending in the United States and other countries.

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# Preface

## *Intended Audience*

The Echo<sup>®</sup> Dose-Response program is designed for use by laboratory personnel who are familiar with preparing microplates for IC50 analyses. This software application automates sample transfer and multiple dilution process to produce the final assay microplates.

## *Software Requirements*

To use the Echo Dose-Response application, the following items are required:

- Echo 5XX Liquid Handler system using software version 2.1 or later.
- License key from Labcyte, Inc.
- Computer system meeting the following requirements:
  - Operating system:** Microsoft<sup>®</sup> Windows<sup>®</sup> XP SP2 or later; .Net Framework 2.0
  - CPU:** Intel<sup>®</sup> Pentium<sup>®</sup> 4 or later (2GHz or greater)
  - Memory:** 512 MB or higher
  - Network connection:** 10/100/1000 BaseT
  - Network protocol:** TCP/IP
  - Hard drive storage:** 200 MB
  - Video:** 1024 X 768 resolution, 256 colors or better

The Echo Dose-Response application works closely with the Echo 55X client software and should be installed on the same client PC.

## *Manual Organization*

This manual is provided on CD and accompanies the Echo Dose-Response installation CDs. Software procedures and screen descriptions in this manual are also available in the Help section of the Echo Dose-Response application.

The Echo Dose-Response user manual is organized as follows:

- Chapter 1, Introduction
- Chapter 2, Getting Started
- Chapter 3, Software Operation
- Chapter 4, Software Reference
- Appendix, Sample Transfer Protocols

## ***Manual Conventions***

Some of the text in this manual uses special formatting to indicate emphasis or keystrokes. The text conventions are described below:

<b>Convention</b>	<b>Example</b>	<b>Meaning</b>
Bold text	Press the <b>Next</b> button.	Indicates an on-screen button, label, menu title, or menu item.
Courier	<code>client.txt</code> , <code>client.old2.txt</code>	Indicates a display output, printed output, keyboard input, or file name.
Blue, underlined	<a href="#">Software installation</a>	Signifies a hyperlink to a topic.
Quotation marks and italics	See " <i>Set up Preferences</i> " on page 14.	Refers you to a topic elsewhere in the manual. Usually includes a hyperlink.

# 1 Introduction

The Labcyte Echo Dose-Response software automates the tedious process of preparing active compound concentrations for IC50 Dose-Response analyses. This software works seamlessly with the Echo Liquid Handler to pick up nanoliter quantities of compound from the source plate, and then transfer them to the assay plates.

Laboratory time and expense are reduced through the following key features:

- Direct transfer from the library plate to assay plate in concentrations required for the dose-response analyses.
- Automation of the transfer process, thus increasing accuracy and eliminating cumulated error during serial dilutions.
- Significant reduction in compound and DMSO volume by using microplates instead of test tubes. Dilution can be performed in 96-, 384-, and even 1536-well plates.
- Cost savings by reducing or eliminating the need for pipettor tips, reservoirs, and other labware required for compound dilution and assay plate preparation.

## 1.1 *What is IC50?*

IC50 is the concentration of a compound that causes a 50% inhibition of enzyme activity in vitro\*. This measurement is commonly used as a measure of effectiveness. The smaller the amount needed, the better the compound is as a drug lead.

Another measure of drug efficacy is EC<sub>50</sub>, which represents the concentration of a compound that is required to obtain 50% of the maximum effect in vivo. Other measurements are LD50 and TD50, briefly described below:

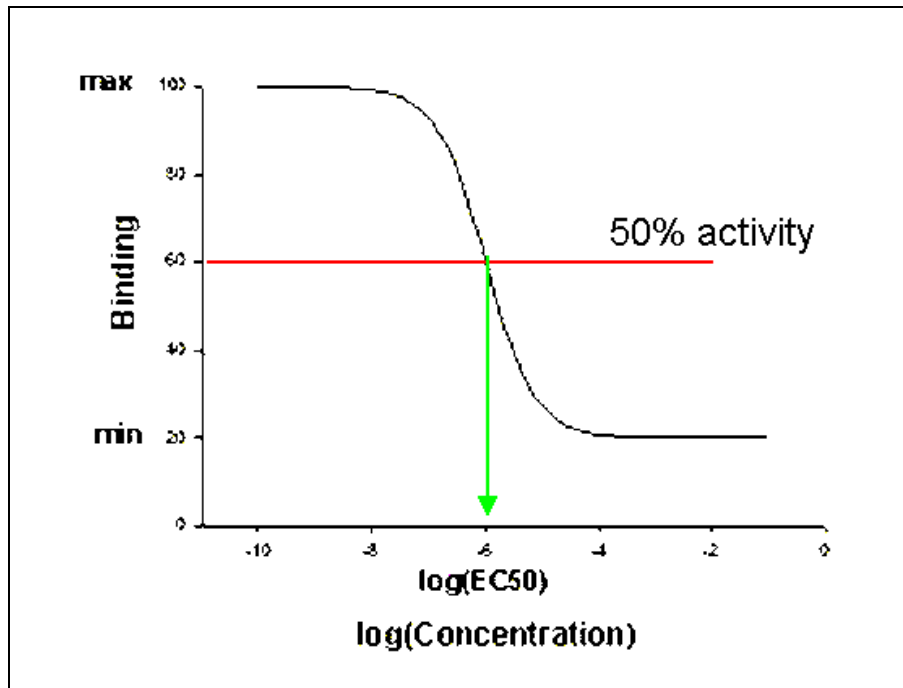
- IC50 is how much is needed to inhibit 50%
- LD50 is how much is needed to kill 50% (Lethal Dose)
- EC50 is how much is needed to cause a 50% effect
- TD50 is how much is needed to cause a toxic effect
- Therapeutic window is the distance between IC50 and TD50

---

\* See <http://www.fda.gov/ohrms/dockets/ac/00/slides/3621s1d/sld036.htm>.

## 1.2 *What is a dose-response curve?*

A **dose-response curve** is a graph that measures the amount of a drug or toxin given to the response of the organism to that drug. The midpoint of the curve represents 50% inhibition.





### 1.3 How does the Echo Dose-Response software fit in?

The process of screening a compound for potential drug development includes the following steps:

1. Test potential compounds against the target antigen. Compounds showing significant activity against the antigen are selected for the next step.

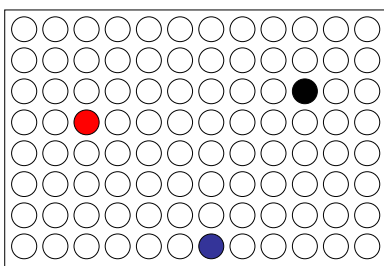


Figure 1-1: Screening plate

2. Perform serial dilutions of the selected compounds. See Figure 1-2. Dilutions are typically calculated at logarithmic levels (for example, 100 mM, 10 mM, 1 mM, 0.1 mM, 0.01 mM). Each dilution represents a point in the dose-response curve.
3. Transfer compound dilutions to assay plates for testing.

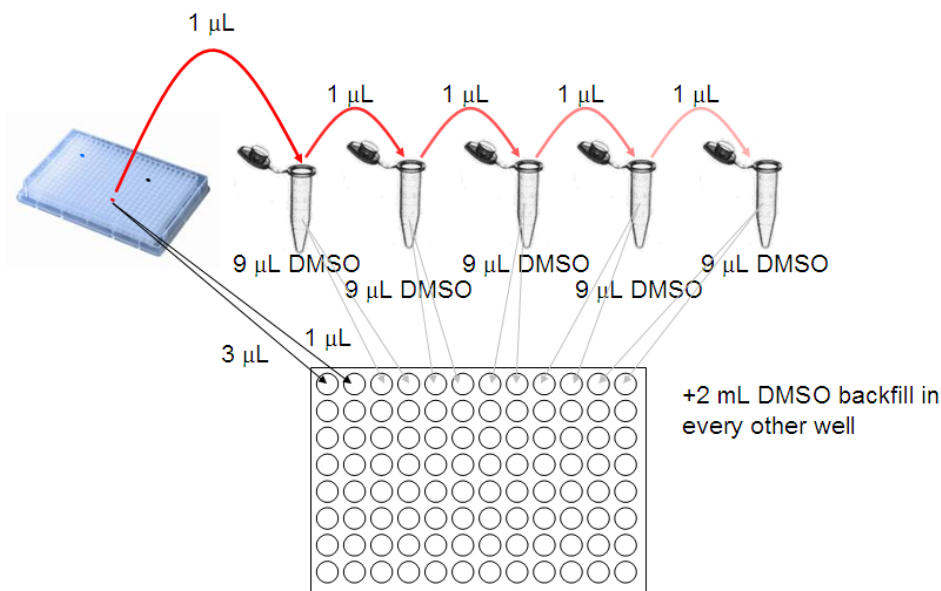


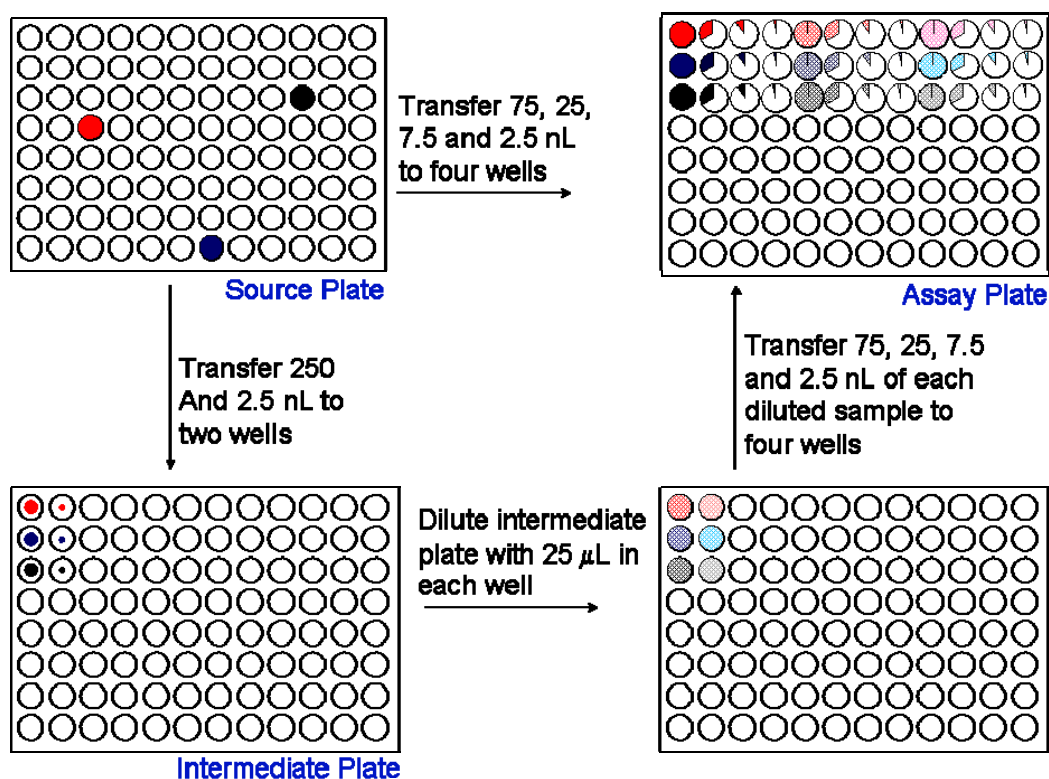
Figure 1-2: Manual dilution and transfer

4. Test the compound dilutions using techniques such as fluorescence or spectrophotometry, and graph the compound concentration against enzyme activity.

**Steps 2 and 3** are the most time-consuming and tedious steps for most laboratories. They can also be quite expensive if the dilution step requires large quantities of compound and diluting agent, such as DMSO. This is the area where the Echo Dose-Response software can help.

The Echo Dose-Response software, together with the Echo 55X liquid handler, can prepare compound concentrations for dose-response analyses by transferring nanoliter volumes of compounds directly into assay plates.

The Echo system can also be used to prepare an intermediate source plate if additional starting concentrations are needed to reach the full range of the curve. See Figure 1-3.



**Figure 1-3: Direct compound transfer for dose-response curves**

In the example above, the Echo system is used to create an intermediate plate with two additional starting concentrations. The system then transfers compounds from the source plate and intermediate plate to the assay plate. The assay plate now has sufficient compound concentrations to create a 12-pt dose-response curve.

All of the steps in this example can be accomplished by the Echo system, using a single Echo dose-response protocol, with a high degree of accuracy and precision. User intervention is held to a minimum.

---

## 1.4 *What are the benefits?*

The Echo Dose-Response software provides the following benefits:

### **Fast**

- ◆ Sets up more than 400 12-point dose-response curves in one hour
- ◆ Generates transfer maps automatically in real time
- ◆ Saves time by efficient transfer to destination plates

### **Flexible**

- ◆ Places control compounds in desired wells specified by the user.
- ◆ Maps the design from any plate to any plate type
- ◆ Provides replicates on the same or separate assay plates
- ◆ Allows user-defined curve orientation
- ◆ Processes dose-response curves from a hit list
- ◆ Allows the output files to be run-specific or destination plate-specific
- ◆ Generates output files in XML, CSV and TXT format

### **Accurate**

- ◆ Eliminates accumulated error from serial dilution
- ◆ Automates manual steps
- ◆ Removes washing steps
- ◆ Maintains survey history

## 2 Getting Started

This chapter describes the basic tasks to start, set up, and finally exit the software.

### 2.1 Software Startup

To start the Echo Dose-Response software:

1. Open the **Start** menu.
2. Select All Programs (or Programs) -> Labcyte -> Echo Dose-Response.
3. Select the **Echo Dose-Response** program.

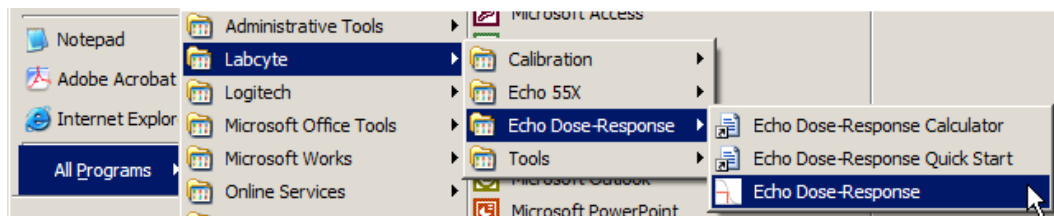



Figure 2-1: Location of Echo Dose-Response Software

**Hint:** To create a shortcut to the Echo-Dose-Response software on your computer desktop, press and hold the **Control** key while you drag the Echo Dose-Response icon  to the desktop.

The software begins with the *Connect to Instrument* screen.

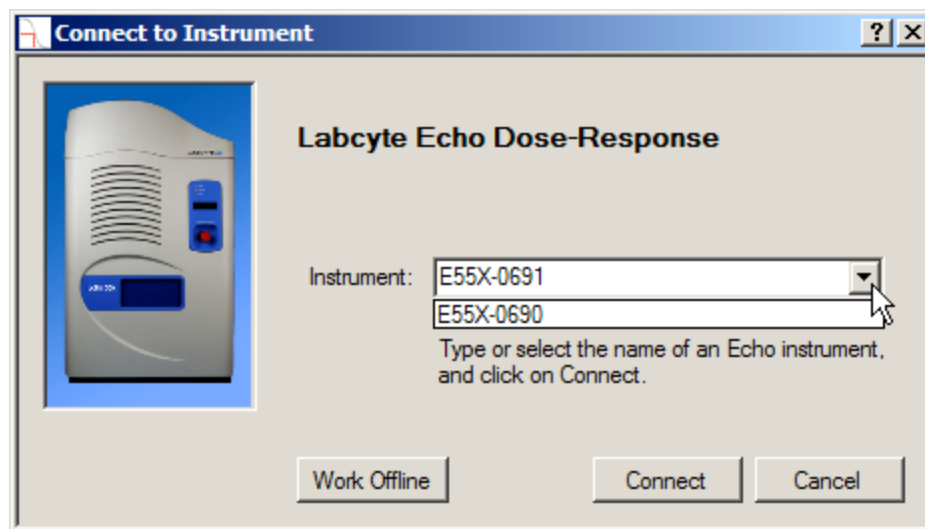
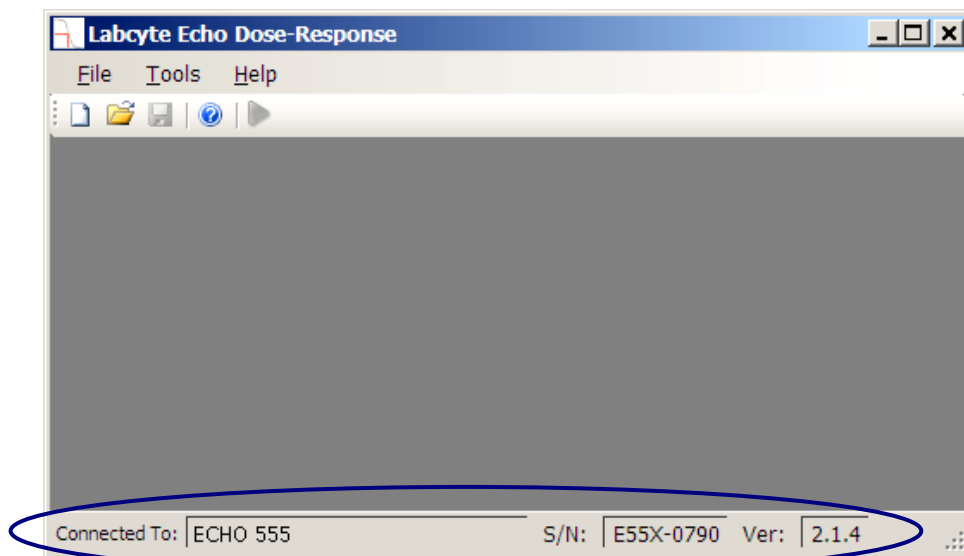


Figure 2-2: Connect to Instrument screen

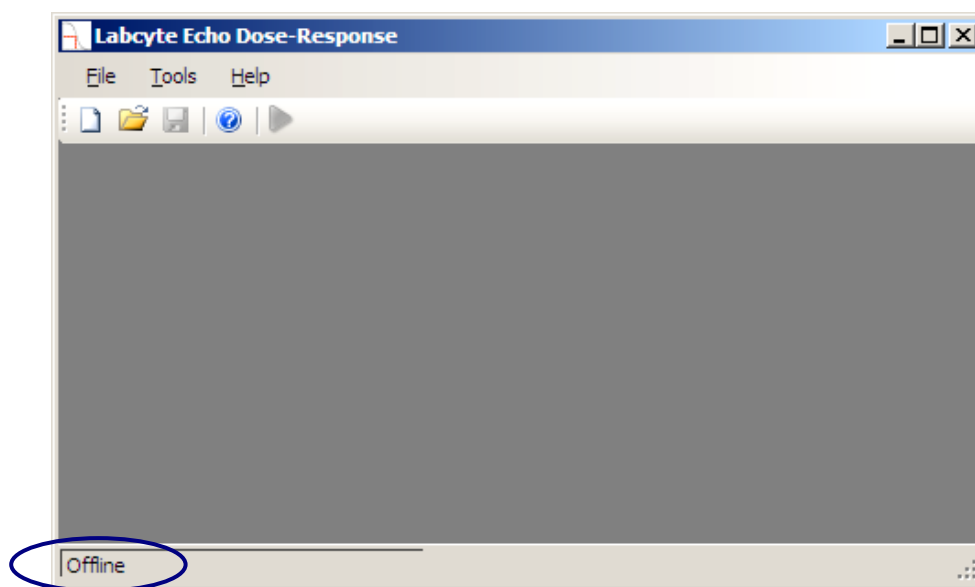
4. Select one of the following start options:

**Connect:** Select an Echo liquid handler from the *Instrument* menu and click **Connect**. The Main Window opens with the connection status instrument information at the bottom of the screen. This option enables you to execute the compound transfer as soon as the transfer protocol is ready. See Figure 2-3.



**Figure 2-3: Dose-Response software connected to Echo instrument**

**Work Offline:** Click **Work Offline**. The Main Window opens with the connection status "offline" at the bottom of the screen. See Figure 2-4. This option enables you to create a transfer protocol and run the transfer simulation without connecting to the Echo liquid handler.



**Figure 2-4: Echo Dose-Response software offline**

## 2.2 Documentation


In addition to this manual, the Echo Dose-Response software comes with the following useful documents:

- Online Help
- Quick Start Guide
- IC50 Curve Calculator

### Online Help

Online Help is available in the Echo Dose-Response software. It contains screen descriptions and procedures.

You can view online Help by using one of the following methods in the software:

- Click the **Help** icon  or **Help** menu in the software toolbar to view the complete Help file.

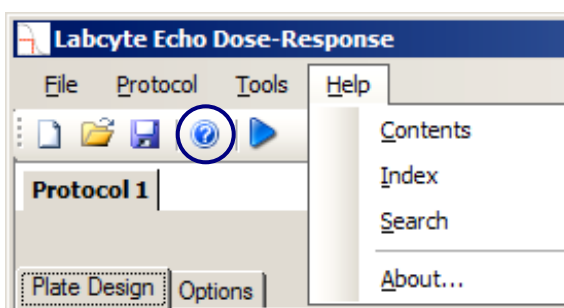



Figure 2-5: Access to Help from the Toolbar

- Click the **Help** icon  in the upper right corner of individual software screens to view the Help page for that screen.

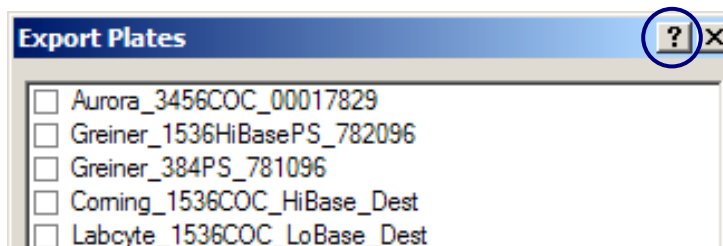


Figure 2-6: Access to Help from the software screen

- Press the **F1** key on the computer keyboard to view the Help page for the displayed screen that does not have a Help icon.

## Quick Start Guide

The Echo Dose-Response Quick Start Guide describes the capabilities of the Echo Dose-Response software and takes you through a sample protocol.

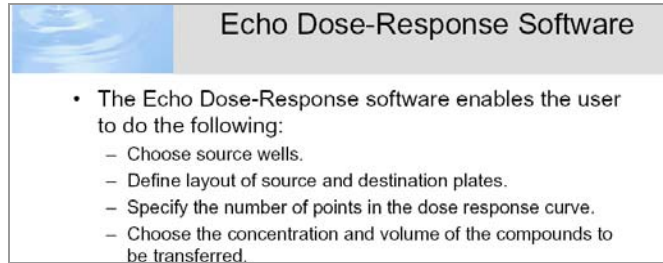


Figure 2-7: Quick Start Guide

The Quick Start Guide can be found in the Echo Dose-Response directory. You will need Adobe Acrobat Reader to open this document.

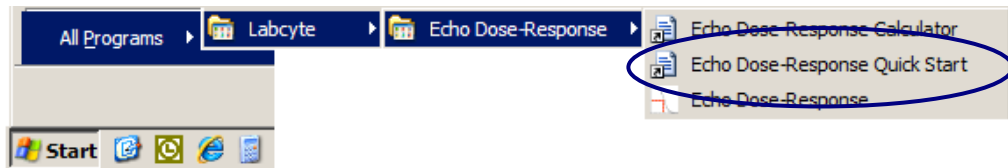


Figure 2-8: Location of Quick Start Guide

## IC50 Calculator

The Echo Dose-Response Calculator is a spreadsheet that contains sample IC50 curve calculations. It includes interactive fields to help you determine volume requirements for compound and DMSO.

What is the concentration of your source solution?

What is the final volume of your assay?

I can tell you how to set up the following IC<sub>50</sub> analyses for you with your Dose-Response software:

- 12-Point, 5.48-Log
- 6-Point, 5-Log (low DMSO concentration)
- 9-Point, 4.82-Log (halving concentrations, low DMSO concentration)
- 19-Point, 5.42-Log (uses more sample)

---

I can set up a 12-point, six-log, IC<sub>50</sub> analysis for compounds of your choice at these concentrations:

	Highest concentration						Lowest concentration					
well number	1	2	3	4	5	6	7	8	9	10	11	12
nL added	75	25	7.5	2.5	75	25	7.5	2.5	75	25	7.5	2.5
μM	1.88E+01	6.25E+00	1.88E+00	6.25E-01	1.88E-01	6.25E-02	1.88E-02	6.25E-03	1.88E-03	6.25E-04	1.88E-04	6.25E-05
% DMSO	0.3750%	0.1250%	0.0375%	0.0125%	0.3750%	0.1250%	0.0375%	0.0125%	0.3750%	0.1250%	0.0375%	0.0125%

The highest concentration of DMSO is  Is this DMSO concentration too high? You can reduce the concentration of DMSO by increasing y

Total sample used = 75+25+7.5+2.5+250+2.5 = 362.5 nL

Sample used for triplicate analysis = 3X(75+25+7.5+2.5) +250+2.5 = 582.5 nL

Total DMSO used, with backfill=25000+25000+3X50+3X67.5+3X72.5=50.57 μL (51.71 if triplicate)

Total DMSO used, no backfill=50 μL

Stock (source)  
1/100 Stock 250 nL/25 μL  
1/10,000 Stock 2.5 nL/25 μL

Figure 2-9: I50 Calculator

The Echo Dose-Response Calculator can be found in the Echo Dose-Response directory. See previous Figure 2-8. You will need Microsoft® Excel to open this document.

## 2.3 Main Window

When you open a new or existing protocol, the *Main Window* displays the *Plate Design* window in the left pane and workflow diagram in the right pane. The *workflow diagram* serves as a guideline to setting up the microplates in the protocol.

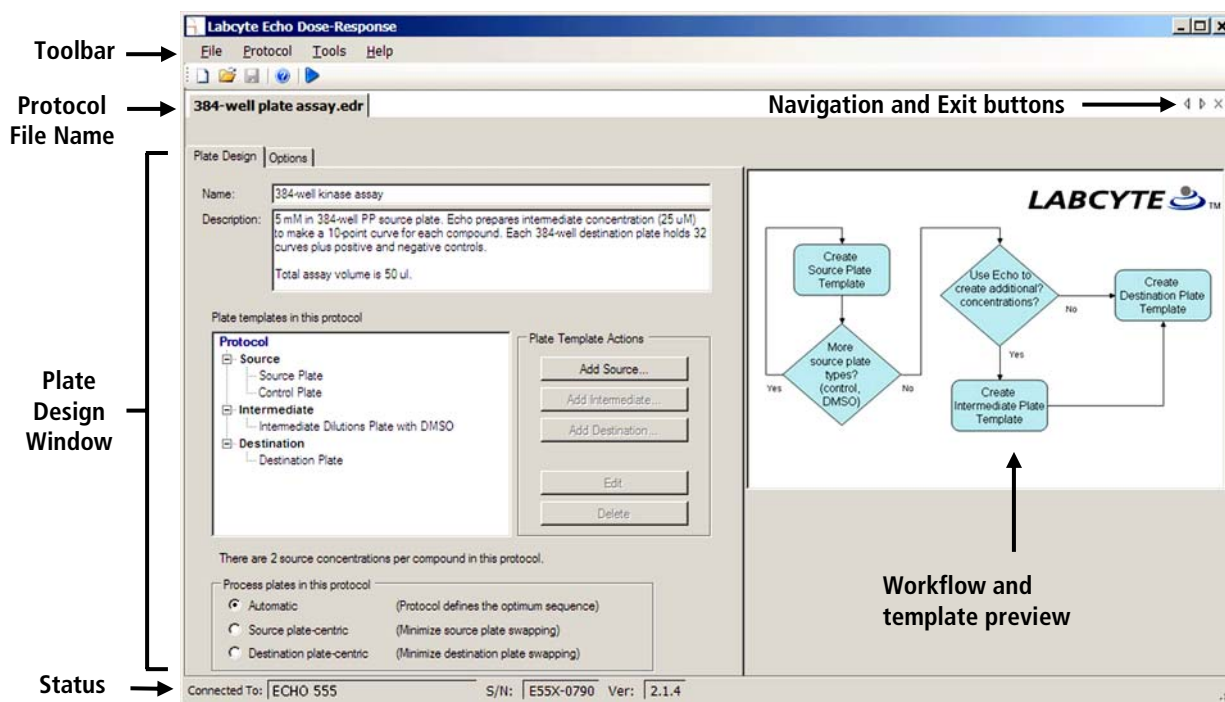


Figure 2-10: Main Window

### Screen Components

The components in the *Main Window* are described below.

**Toolbar:** The toolbar contains software menus with functions to open and save transfer protocols, set up the software, define labware, run the protocol, customize protocol options, and display the online Help. The *icons* in the toolbar provide shortcuts to the most frequently-used functions, such as the *Run Protocol* function. For a detailed description, see "*Toolbar*" on page 65.

**Protocol file name:** The protocol file name identifies your transfer protocol in the computer's directory. The software uses an ".edr" file extension and stores the file in the default directory: C:\Labcyte\Echo\Protocols. You can store your protocols in different directories.

**Navigation and Exit buttons:** The left and right arrow heads (◀ ▶) become active when you have too many protocols open to view all of the file names. The arrows shift the protocol tabs left or right to display the file names. The **Exit** icon (×) closes the displayed protocol.



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**Plate Design window:** The *Plate Design* window contains all of the required protocol data necessary to perform the compound transfer. For a detailed description, see "*Plate Design*" on page 51.

**Options window:** The *Options* window (behind the *Plate Design* window) displays protocol-specific options, such as survey history, output file type, and report formats. For a detailed description, see "*Protocol Options*" on page 79.

**Workflow and template preview:** The workflow diagram is displayed whenever a protocol is open. It serves as a reference tool for planning the protocol. When you select any template in the template tree (left pane), the plate design for the template is displayed in the right pane.

**Status bar:** The status bar displays Echo connection information, such as instrument name and serial number, and the version of Echo client software that is running.

**Navigation and Exit buttons:** The left and right arrow heads (◀ ▶) become active when you have too many protocols open to view all of the file names. The arrows shift the protocol tabs left or right to display the file names. The **Exit** icon (✕) closes the displayed protocol.

## 2.4 Software Setup

Your Echo Dose-Response software needs very little setup. It comes with default settings, such as output file type and report format, that can be used.

If you want to change these settings, refer to the tasks listed below; otherwise continue to the next section, "*Software Overview*", on page 17.

### 2.4.1 Activate Instrument

To run the Echo Dose-Response software on an Echo liquid handler, you will need to obtain a software license key from Labcyte.

- If you purchased the Echo liquid handler and Echo Dose-Response software at the same time, your instrument will be delivered with the key pre-installed.
- If you purchased the Echo Dose-Response software separately, then you will have to perform this task.

To activate the instrument:

1. Start the Echo Dose-Response software.
2. In the *Connect to Instrument* screen, select an instrument that you will be using with the Echo Dose-Response software and click the **Connect** button.

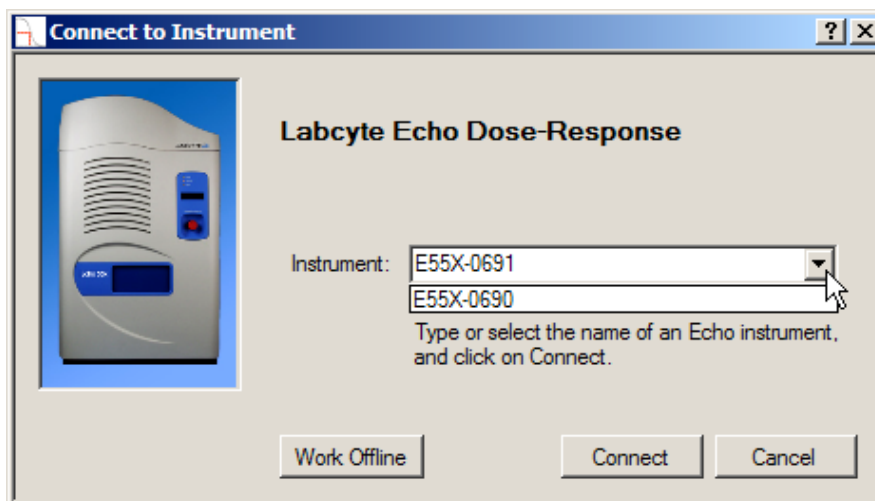
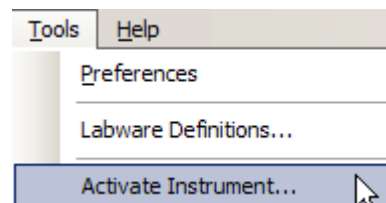


Figure 2-11: Connect to instrument

3. Go to the *Tools* menu and select the **Activate Instrument** option.



4. Enter the license key. The license key is an encrypted block of text. You can insert the license key into the *Activate Instrument* window in one of the following ways:
  - ◆ Copy the license key from the file you received from Labcyte and paste it into the *License Key* text box.
  - ◆ Click the **Open License Key File** button and browse for the license key file.

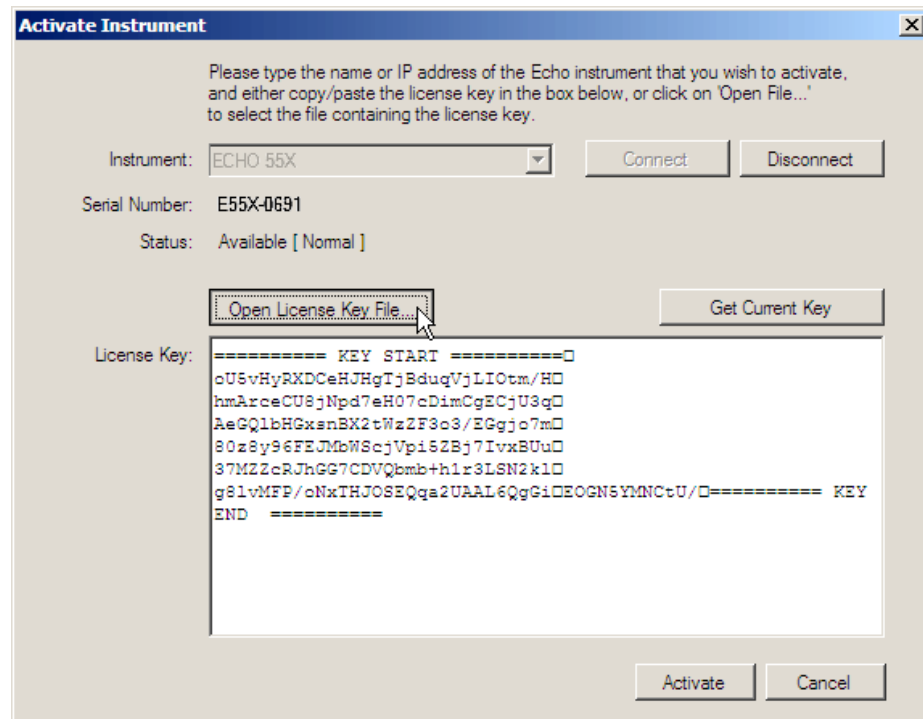


Figure 2-12: Activating instrument

**Note:** The **Get Current Key** function is a useful troubleshooting tool to verify that the key is written correctly to the instrument. When you click the **Get Current Key** button, the software will search for the license key and insert it into the *License Key* text box. Send the encrypted key to Labcyte to determine if the license key is corrupted or if it applies to a different instrument.

5. Click the **Activate** button.

The Echo liquid handler is now activated. You will not have to perform this procedure again unless you need to activate a different instrument.

## 2.4.2 Set up preferences

Your laboratory applications for dose-response analyses may require the transfer data in a specific file and data format. The Echo Dose-Response software provides transfer data in three file formats (csv, xml, and txt) with reporting options that can be customized to meet your needs.

1. Open the *Tools* menu and select **Preferences**.

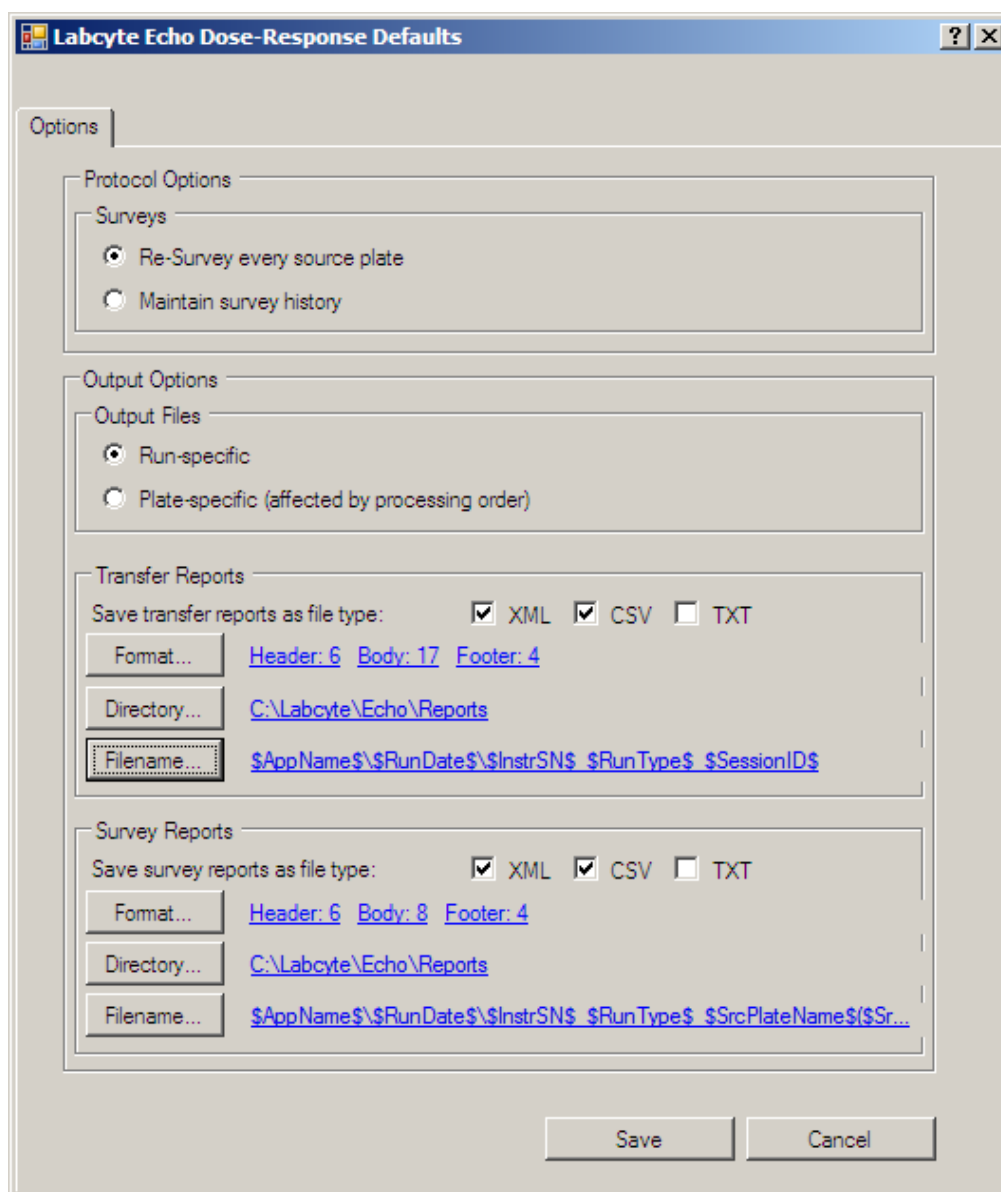
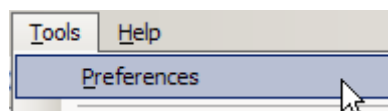


Figure 2-13: Preferences/Options screen

- 
2. Select **Maintain survey history** if you want to re-use the current plate survey for more than one transfer operation for a specified period of time. For more information, go to "*Survey History*" on page 80.
  3. Select **Run-specific** or **Plate-specific** output files.
    - ◆ **Run-specific:** Store all transfer report data in one file.
    - ◆ **Plate-specific:** Store the transfer report data in separate files, based on source or destination plates (depending on which process order you specified in the *Plate Design* window)
  4. Customize transfer and survey reports:
    - ◆ **File type:** Select XML, CSV, and/or TXT file formats.
    - ◆ **Format:** Customize the transfer report header, body, and footer.
    - ◆ **Directory:** Change the directory location for the transfer report.
    - ◆ **Filename:** Change the file name for the transfer report

These options will be used as default settings for all transfer protocols. If you want to change the options for an individual protocol, use the **Options** tab. See "*Preferences*" on page 87 or "*Protocol Options*" on page 79 for details.

The software is set up. You are ready to create and run a transfer protocol.

## 2.5 Software Shutdown

You can exit the Echo Dose-Response software in the following ways:

- Open the *File* menu and select **Exit**.

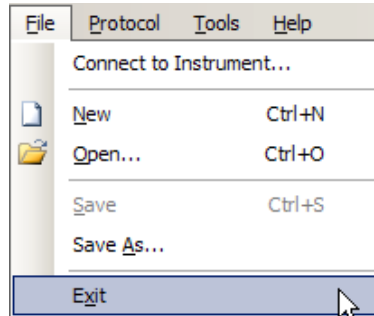


Figure 2-14: Exit function in the File menu

- Click the **Close** icon in the software window.

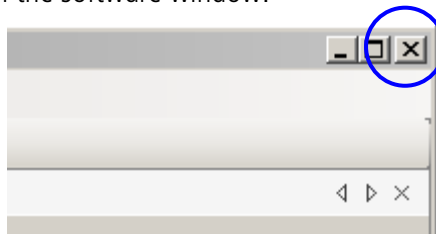


Figure 2-15: Close Window icon

The software will prompt you to save your protocol before it shuts down.

## 3 Software Operation

The best way to describe the features of the Echo Dose-Response software is to take you through the steps to create and execute a transfer protocol.

### What is a transfer protocol?

In the Echo Dose-Response software, the transfer protocol is a set of plate templates and transfer options that help you design the dose-response curves (up to 24-point) in the assay plates.

The software automatically creates the optimal transfer sequence that is sent to the Echo liquid handler to execute the direct compound transfer to assay plates.

After the transfer is complete, you can retrieve custom reports that document the transfer run and provide well data for sample tracking.

### Sample transfer protocol

The sample transfer protocol in this chapter uses a single column of compound to illustrate a simple compound transfer. This protocol will use the original compound concentration in a source plate, as well as two compound dilutions in an intermediate plate, to create an assay plate that can generate a 12-point IC50 curve.

Throughout this overview, there will be references to specific topics in the *Software Reference* chapter, beginning on page 50, which can provide additional details.

The steps to creating and executing a transfer are listed:

1. **Plan the Curve Data:** provided by the user to create the protocol.
2. **Set up the Transfer Protocol:** created by the user in the Echo Dose-response software.
3. **Set Up Plates:** performed by the user before running the protocol.
4. **Specify the order of transferring and diluting compound:** performed by the Echo Dose-response software and Echo 55X liquid handler.
5. **Run the Protocol:** performed by the Echo Dose-response software and Echo 55X liquid handler.
6. **Manage Transfer Data:** generated by the Echo Dose-response software, based on user settings.

Each step contains screen descriptions and procedures. By the end of this section, you will have a good basic understanding of the Echo Dose-Response software.

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**Note:** After you have read through the protocol in this section, review the sample protocol in the *Appendix*. It describes additional software options that may be more applicable to your needs.

---

### 3.1 *Plan the Curve Data*

Before you start the Echo Dose-Response program, have the following information ready:

- Initial concentrations of your compound
- Number of compounds
- Location of controls, if they are used (control samples can be on the compound source plate, separate source plate, or destination plate)
- Layout for the source plate
- Layout for the intermediate plate (if additional compound concentrations are needed)
- Final assay volume
- Number of points needed for the IC50 curve

You may already have a calculation utility to help you with the IC50 curve. If not, try the Echo Dose-Response calculator in the Echo Dose-Response directory. The calculator contains sample curve data that shows the volume of compound and DMSO needed for different IC50 analyses.

Also, see the sample protocols included with your software for examples to follow.

For this sample protocol, the following data will be used:

Starting concentration	Original compound at 5 mM
Layout for the compound source plate	16 compound wells (two columns in 384-well plate)
Layout for the control source plate	32 control wells (two columns in 384-well plate)
Layout for the intermediate plate	Two dilutions of stock compound in 384-well plate: 1/100 1/10,000
Number of points needed to plot the IC50 curve	12-point curve
Assay reagents added to each well	25 $\mu$ L



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## **3.2 *Set up the Transfer Protocol***

The Echo Dose-Response software is very flexible. It enables you to pick compounds from any well position in your source plate and transfer them into your final assay plates to generate all the compound concentrations necessary to create an IC50 curve. If you need to expand the range of the IC50 curve, the software provides an intermediate plate template to create additional source concentrations.

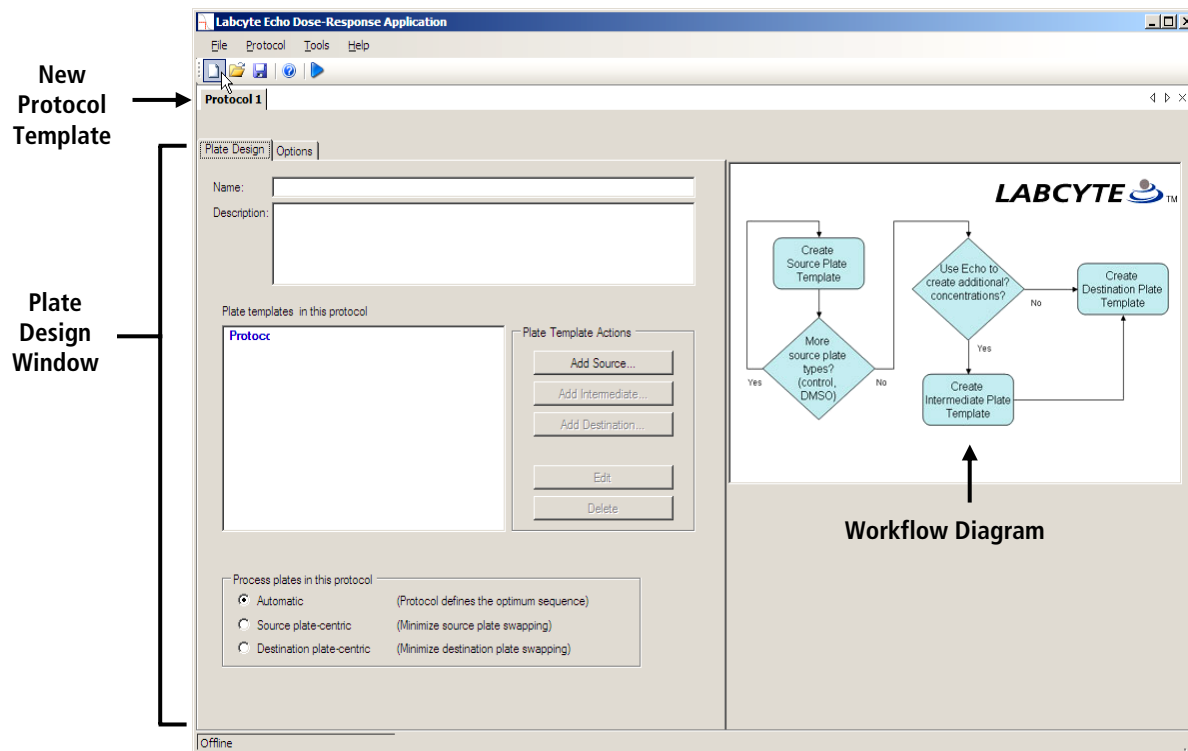
The steps to create the sample transfer protocol are listed below and explained in detail in the subsequent sections:

1. Start a new protocol. Enter the name and description.
2. Create the source plate template. Assign compound concentration.
3. Create the intermediate plate template. Create compound intermediate concentrations.
4. Create the destination plate template: Enter the number of data points, compound transfer volume, assay volume, number of replicates, and fill direction.
5. Specify the order of transferring compound.
6. Save the protocol.

### 3.2.1 Start a new protocol

1. Click the new protocol icon  in the toolbar at the top of the screen, or select **New** from the *File* menu to open a new Protocol template.

The new protocol template opens in the *Main Window*. The *Plate Design* window is displayed in the left pane and workflow diagram in the right pane.



**Figure 3-1: Main Window**

The new protocol is temporarily labeled “Protocol 1” until you save it.

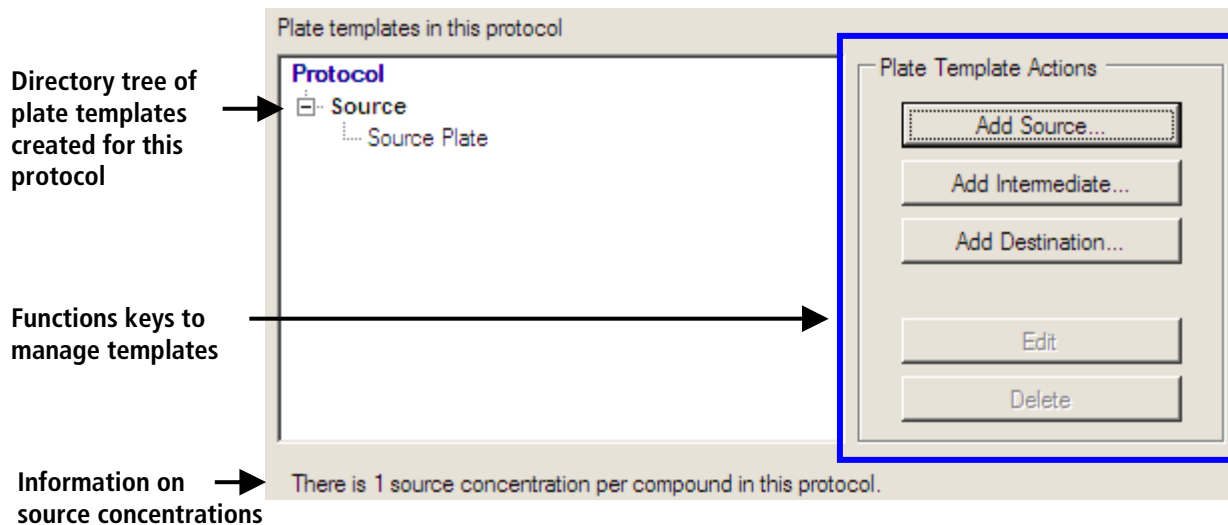
2. **Enter the name and description.**

Type the name and description of the protocol in the text fields. The description should have sufficient information to serve as a reminder the next time you open this protocol.

**Figure 3-2: Name and description of new protocol**

### 3.2.2 Create the source plate template

The *Plate Template Actions* box contains the function keys to design the plate templates and add them to the protocol.



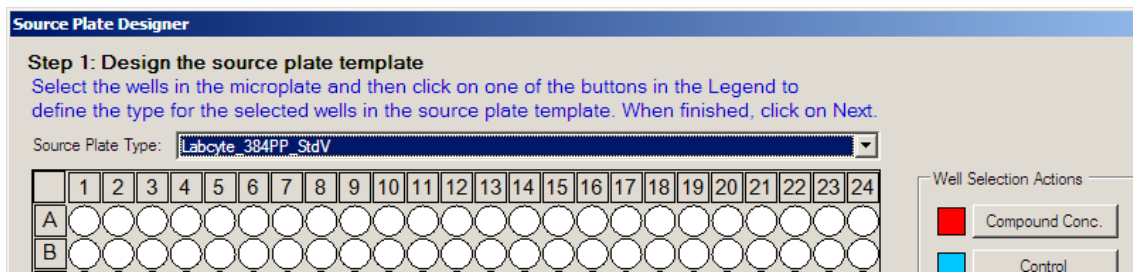
**Figure 3-3: Plate template tree and function keys**

To create the source plate template:

1. Click the **Add Source** button in the *Plate Template Actions* box to start the *Source Plate Designer* wizard.

The *Source Plate Designer* wizard is a series of software screens, with instructions, that take you through each step needed to create the source plate template.

2. Select the **source plate** from the *Source Plate Type* menu. For example, select **Labcyte\_384PP\_StdV**. See Figure 3-4.

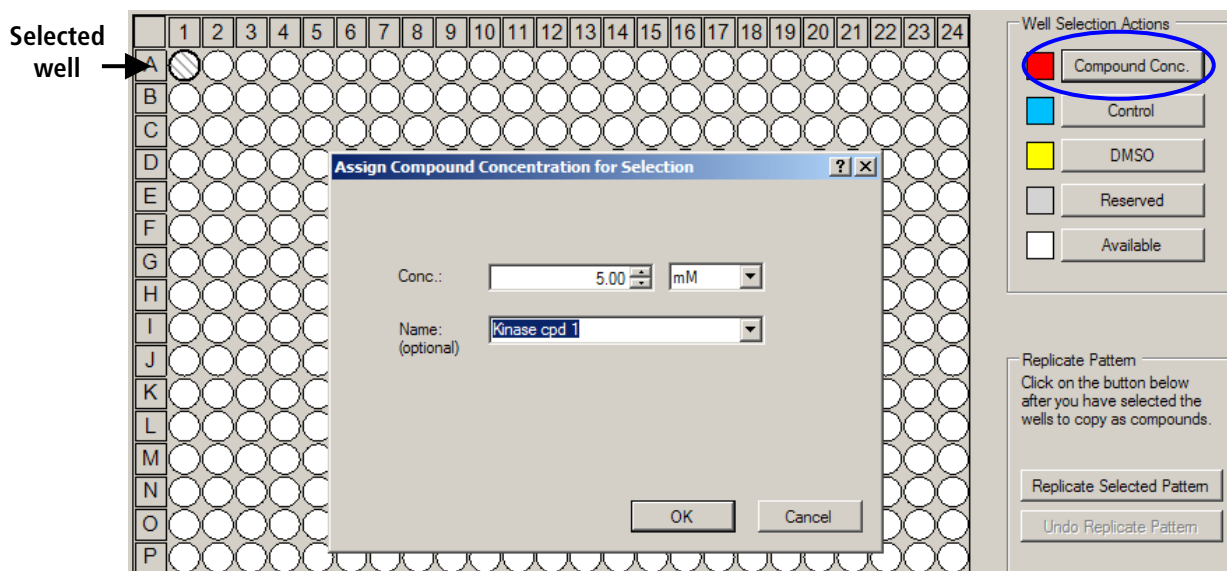


**Figure 3-4: Source Plate Designer window, step 1**

**Note:** The source plate must be an Echo-qualified plate.

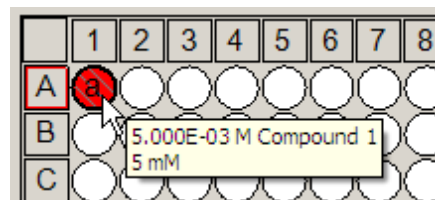
3. Assign compounds to the source wells.
  - a. Select the well that will contain the first compound. The selected well is displayed with diagonal lines. See Figure 3-5.
  - b. Click the **Compound Conc.** button and enter the compound concentration and compound name in the prompt box. In this example, enter **5 mM** and **Compound 1**. See Figure 3-5.

**Note:** If you plan to replicate and number this compound, do not enter a compound name. You will enter the compound name and starting number in the *Replicate Pattern* function.



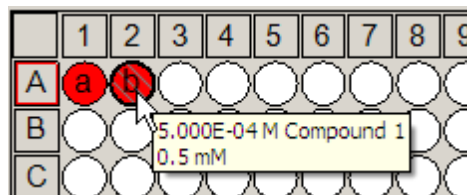
**Figure 3-5: Compound wells in source plate**

- c. Click **OK** to close the prompt box. The assigned well changes to red and is labeled "a". The "a" signifies the first concentration of the compound.



**Hint:** You can move the cursor over the assigned well to view the compound name and concentration.

- d. Repeat steps a to c for the next compound concentration (0.5 mM). The second concentration of the same compound will be labeled "b", and so on.



4. Replicate the pattern.

To replicate the compound concentrations across a specific portion of the source plate:

- a. Select the wells you do **not** want to use for compounds and click the **Reserved** button to block these wells. Some of the reserved wells will be used for controls and DMSO later on.

**Note:** To select columns, click the first column heading and drag the pointer to the last column heading.

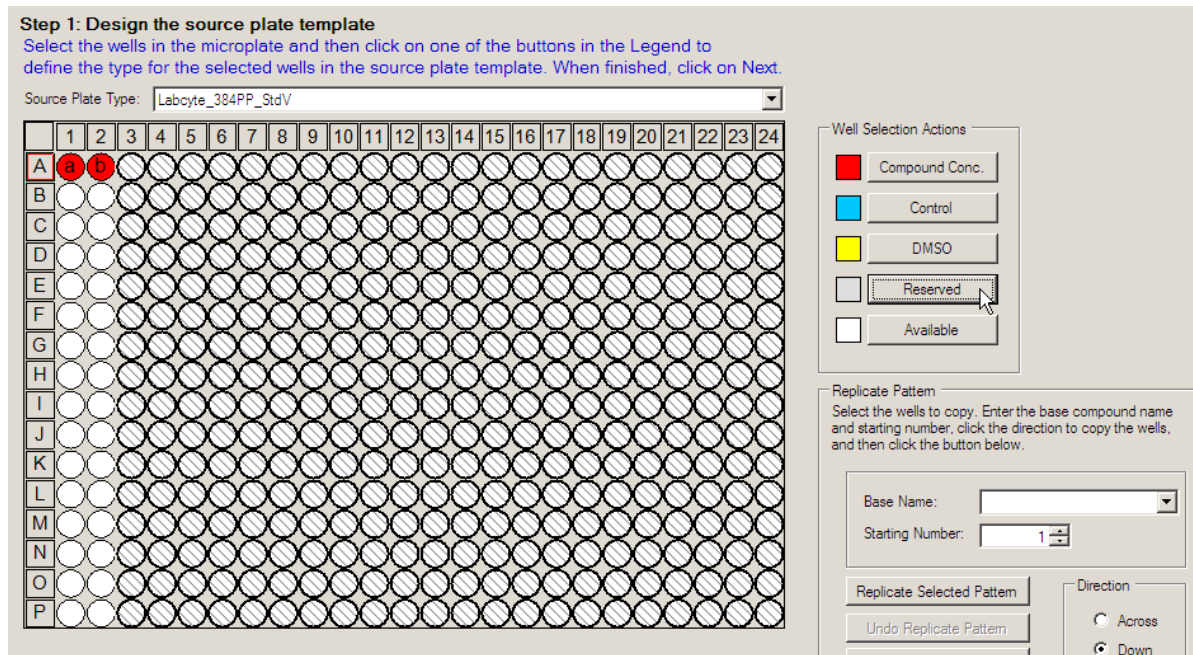


Figure 3-6: Unused wells blocked

- b. Select the wells you want to replicate. The selected wells represent the pattern that will be replicated and numbered.
- c. Enter the base name for the compound, starting number, direction to number the compounds (across or down), and then click **Replicate Selected Pattern**.

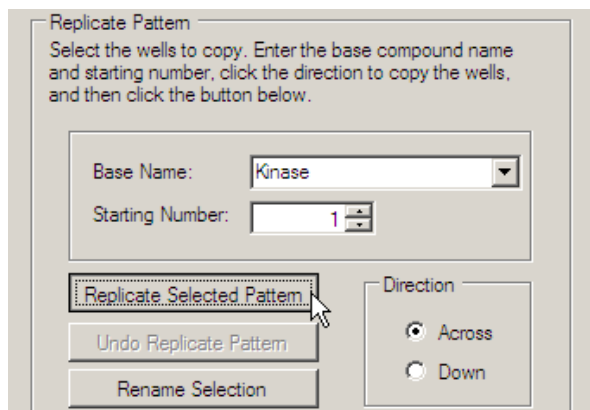


Figure 3-7: Compound name and starting number

The software will replicate and number the compounds in the direction you specified.

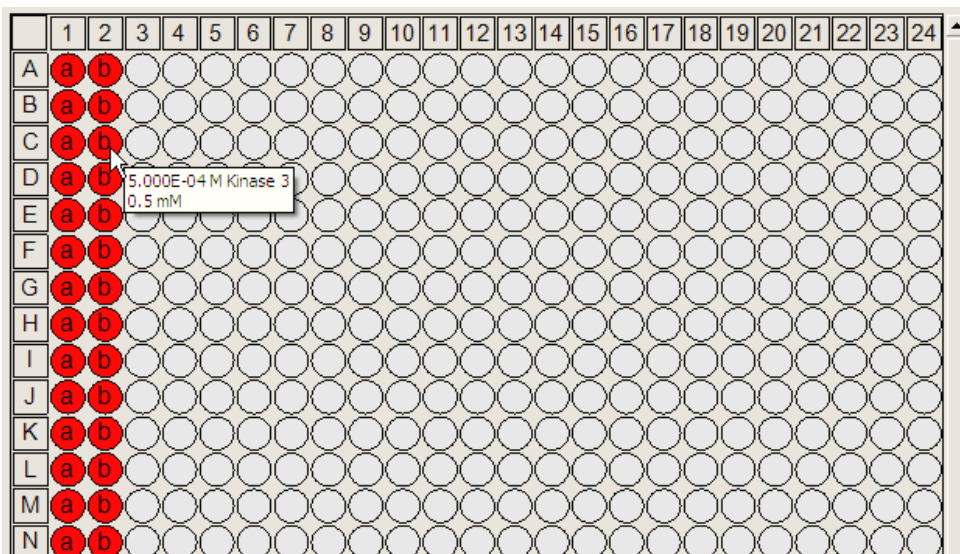


Figure 3-8: Replicate compounds

5. Assign controls and/or DMSO.

◆ To enter controls:

- Select the column (or row) to assign to controls.
- Click the **Control** button.
- Enter the control name in the prompt box and click **OK**. The control wells are highlighted in blue.

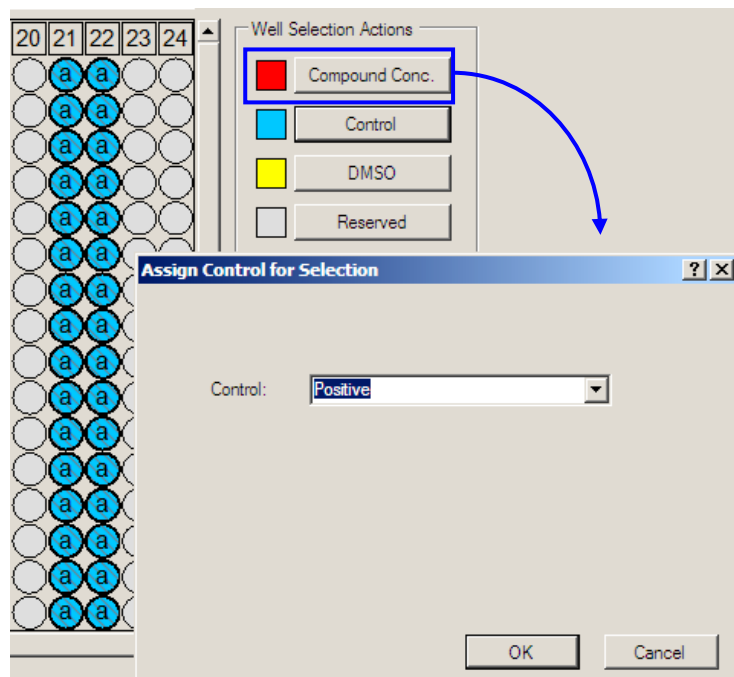
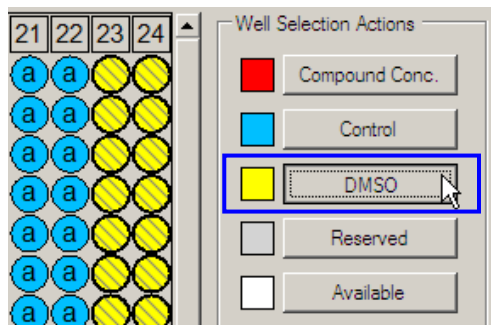


Figure 3-9: Add controls

- ◆ **To enter DMSO wells:**
  - a. Select the column (or row) to assign to DMSO.
  - b. Click the **DMSO** button. The DMSO wells are highlighted in yellow.

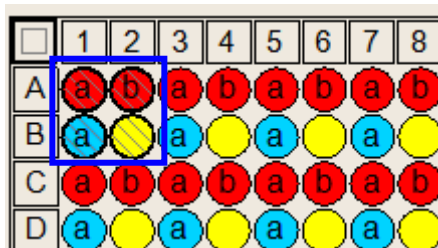


**Figure 3-10: Add DMSO**

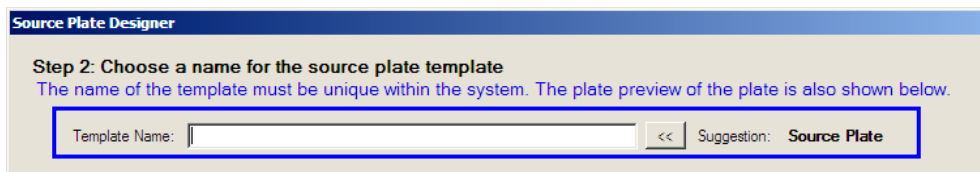
**HINT:** You can include controls and DMSO in the compound replicating pattern.

- a. Assign individual wells to controls and DMSO.
- b. Select the compounds, controls, and DMSO.
- c. Enter the compound name, starting number, direction, and then click **Replicate Pattern**.

Only the compounds will be named and numbered.



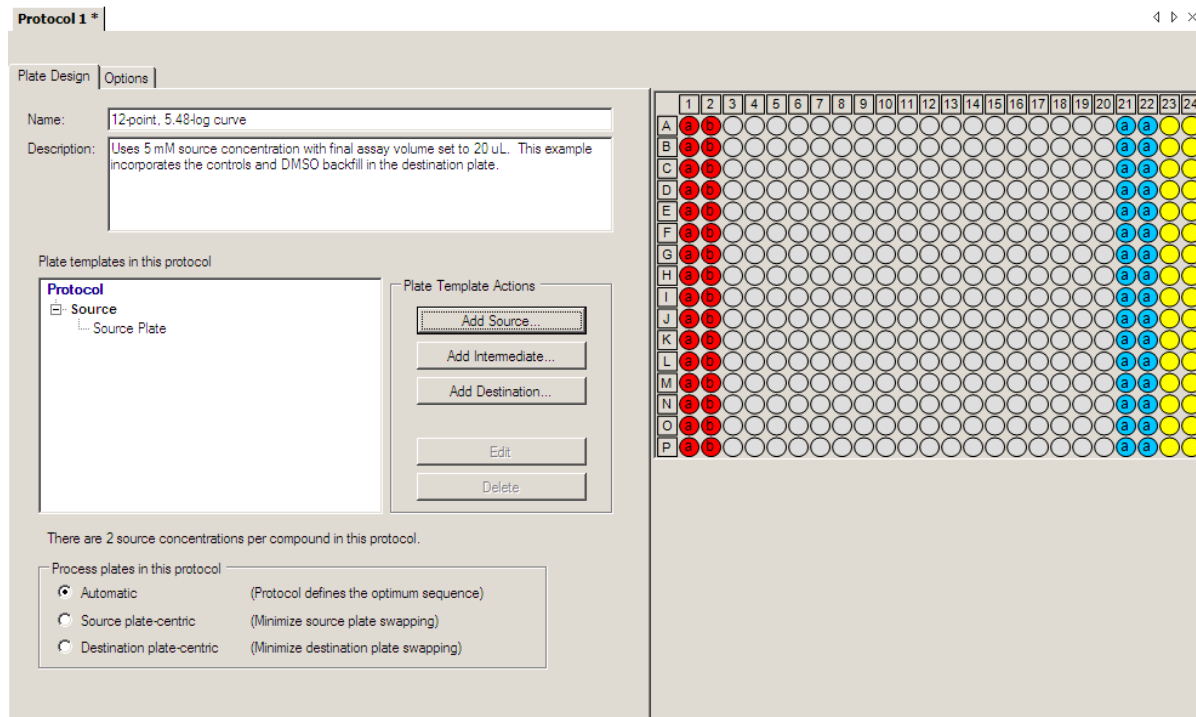
6. Click the **Next** Button and enter the source template name. Type a name in the *Template Name* field or click the left arrow << button to accept the name that is suggested.



**Figure 3-11: Source plate template name**

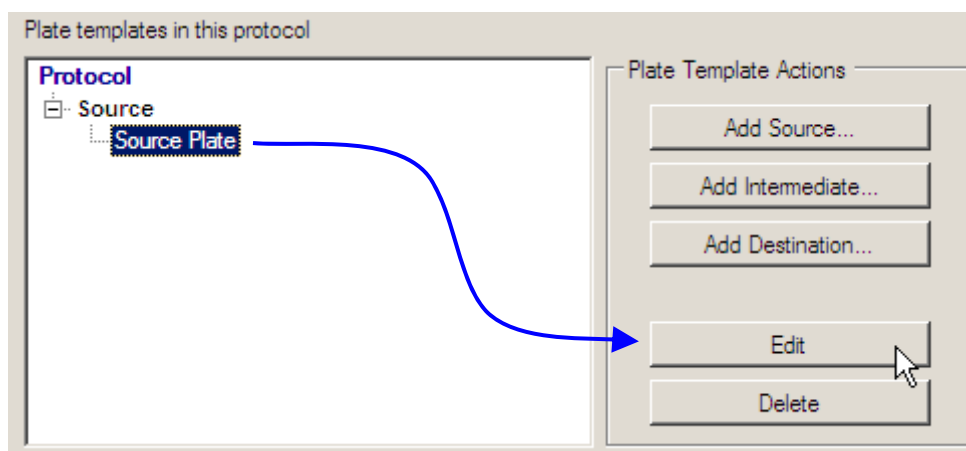
7. Click the **Finish** button to return to the *Plate Design* window.

The source plate definition is complete. The *Plate Design* window displays the design of the source plate in the left pane.



**Figure 3-12: Source plate definition completed**

If you need to edit or delete the source plate template, you can select the source plate template in the protocol tree and click **Edit** to open the *Source Plate Designer* window or **Delete** to remove the source plate.



**Figure 3-13: Source plate template editing**



### 3.2.3 Create intermediate plate template

Typical transfer volumes for an Echo are from 2.5 nL to 250 nL, which translates to 2 logs on an IC50 curve. You can use the intermediate plate to create additional dilutions on an IC50 curve that can span more than five logs.

1. Click the **Add Intermediate** button in the *Plate Design* window to start the *Intermediate Plate Designer* wizard.
2. Select the **source plate** from the *Source Plate Type* menu. For example, select **Labcyte\_384PP\_StdV**.

**Note:** Since the intermediate plate will be used as both a destination plate and source plate, it must be a source plate type.

3. Select **Bulk Fill Direction: Column-wise**. Before assigning wells to DMSO, specify the direction that the assay wells are to be bulk-filled. The fill direction is dependent on the capabilities of your bulk filler.
4. Select the columns of wells that will be reserved for DMSO.
5. Click the **Bulk-Fill DMSO** button and enter fill volume in the prompt box.

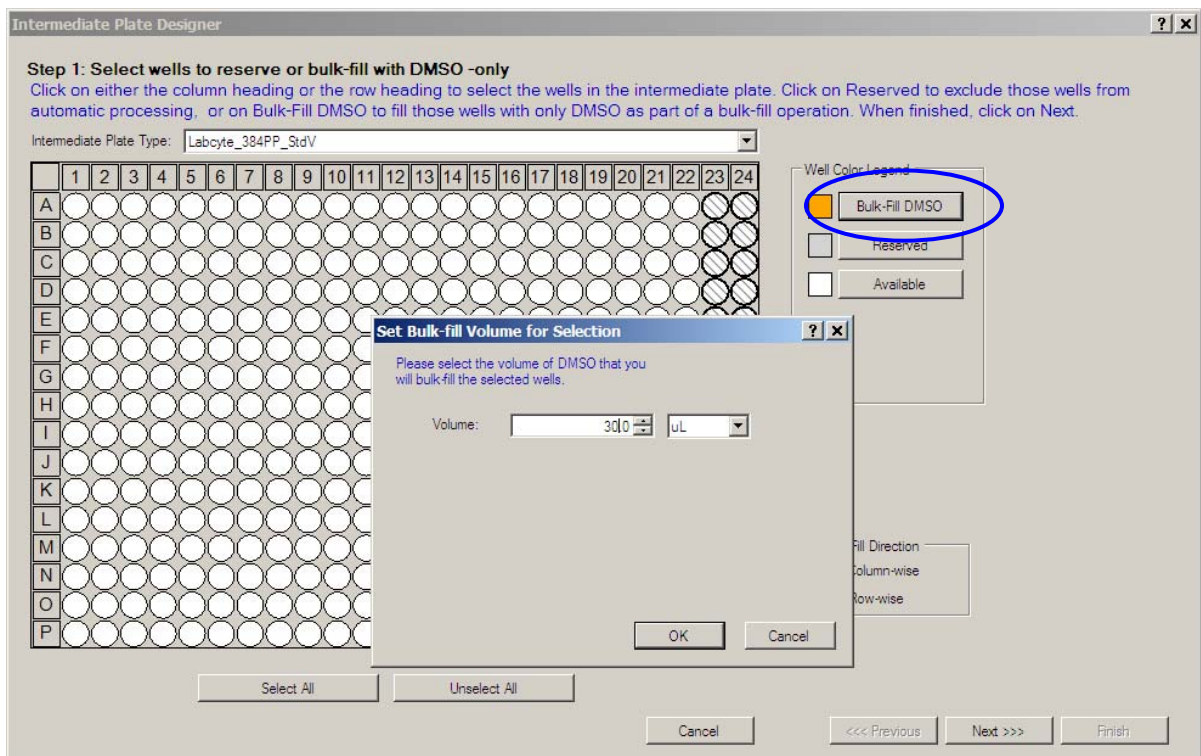
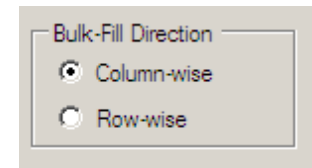


Figure 3-14: DMSO wells in source plate

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**Note:** The selected wells will be used as reservoirs for DMSO only. During plate preparation (or during the run when the bulk-fill screen is displayed) you will fill the intermediate plate with DMSO, either manually or with a bulk-filler. The Echo system will use the DMSO in these wells to backfill the compound wells in the assay plate.

---

6. Click **Next**. The next screen provides fields to calculate the intermediate compound concentrations.
7. Enter the following information:
  - ◆ Enter the number of intermediate concentrations you want to create. In this example, enter **1**. A single concentration field will be displayed. See Figure 3-15.
  - ◆ Select the compound concentration from the **Source Concentration** menu.
  - ◆ Enter the transfer volume of the compound. In this example, enter **25 nL**.
  - ◆ Enter the volume of DMSO you want to bulk fill: **25  $\mu$ L**.  
The software will calculate the final concentration of the compound in the intermediate plate.

Source Concentration	Transfer Volume	Bulk-Fill Volume	New Concentration
5.000E-03 M	25.0 nL	25.00 $\mu$ L	4.995E-06 M

**Figure 3-15: Intermediate concentration**

---

**Note:** The range of the new concentrations you create depends on the working range of the source plate you use. The plate with the widest range is **Labcyte 384PP StdV**, which has a working range from 20  $\mu$ L to 50  $\mu$ L.

For working ranges on other source plates, go to [www.Labcyte.com](http://www.Labcyte.com), click the **Products** menu, select **Consumables**, and open the Web page for Echo-qualified microplates.

---

8. Click the **Next** button and enter the intermediate template name:  
**Intermediate Dilutions Plate**

- Click the **Finish** button to return to the *Plate Design* window.

The software will create additional source concentrations for each compound. The following figure shows 32 wells assigned to the intermediate compound concentration (two concentrations for each compound) and several wells assigned to hold bulk DMSO.

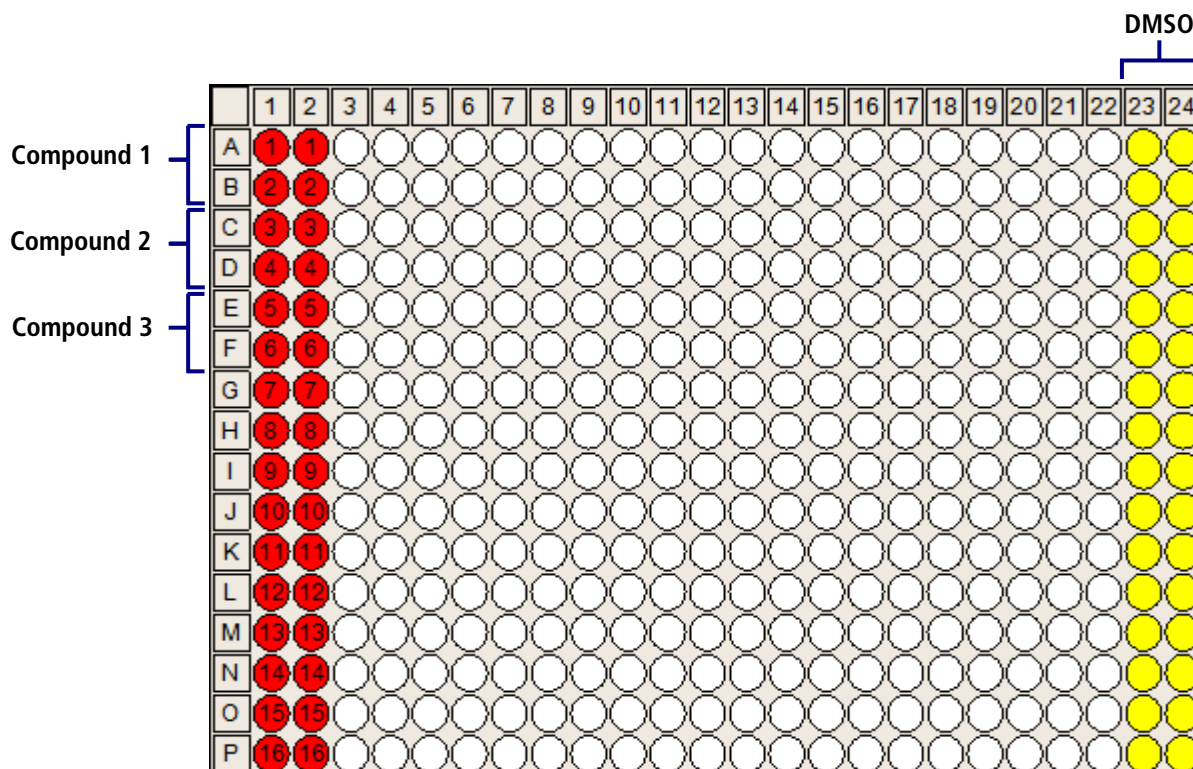


Figure 3-16: Intermediate plate design

The intermediate template is done.

### 3.2.4 Create destination plate template

1. Click the **Add Destination** button in the *Plate Design* window to start the *Destination Plate Designer* wizard.

The destination plate template is similar to the intermediate plate template. You can assign controls to specific wells, assign DMSO-only wells, or block certain wells from being included in the compound transfer.

2. Select the **destination plate** from the *Destination Plate Type* menu. For example, select **Greiner\_384PS\_781096**.

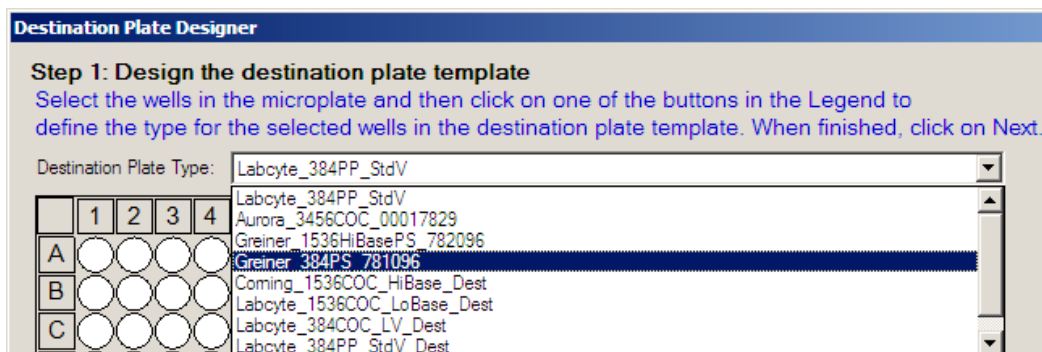


Figure 3-17: Destination plate type menu

**Note:** Since the destination plate will be receiving the liquid transfer, you have a broader range of plate types to choose.

3. Click the **Next** button. The next screen provides fields to calculate the dose-response curve. The data you enter will define the layout of the final assay plate.
4. Enter the number of data points in the *Dose-Response Curve* box (see Figure 3-19):
  - ◆ Specify the *Number of data points per curve*: **13**.  
(12 pt curve plus 1 control)
  - ◆ Select the option **Include Controls in Dropdown Lists**.

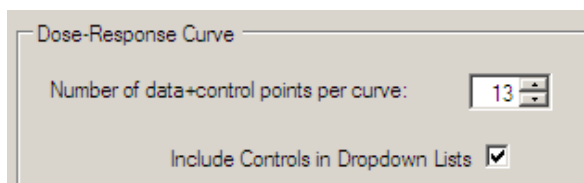


Figure 3-18: Number of data points per curve

The Data Point Curve table will automatically update to display the number of rows corresponding to the number of points in the dose-response curve.

This example appends a control to each curve instead of using separate columns in the plate.

5. Enter the following data in the *Data Point Curve* table (see Figure 3-19):

- ◆ Select the compound concentration from the drop-down menus:

Data points 1 – 4: **5.00E-03**  
 Data points 5 – 8: **4.95E-05**  
 Data points 9 – 12: **5.00E -07**  
 Control name 13: **Positive**

- ◆ Enter the transfer volume for each data point:

Data points 1 – 4: **75, 25, 7.5, 2.5 nL**  
 Data points 5 – 8: **75, 25, 7.5, 2.5 nL**  
 Data points 9 – 12: **75, 25, 7.5, 2.5 nL**  
 Control volume: **75 nL**

#	Source Concentration	Transfer Volume (nL)	Backfill Volume (nL)	Assay Volume (uL)	Final Compound Concentration	Final %DMSO
1	5.000E-03... ▾	75.0	0	25	1.496E-05 M	0.300
2	5.000E-03... ▾	25.0	50	25	4.985E-06 M	0.300
3	5.000E-04... ▾	7.5	67.5	25	1.496E-07 M	0.300
4	5.000E-04... ▾	2.5	72.5	25	4.985E-08 M	0.300
5	5.000E-04... ▾	75.0	0	25	1.496E-06 M	0.300
6	5.000E-04... ▾	25.0	50	25	4.985E-07 M	0.300
7	4.950E-05... ▾	7.5	67.5	25	1.481E-08 M	0.300
8	4.950E-05... ▾	2.5	72.5	25	4.935E-09 M	0.300
9	4.950E-05... ▾	75.0	0	25	1.481E-07 M	0.300
10	4.995E-07... ▾	25.0	50	25	4.980E-10 M	0.300
11	4.995E-07... ▾	7.5	67.5	25	1.494E-10 M	0.300
12	4.995E-07... ▾	2.5	72.5	25	4.980E-11 M	0.300
13	Positive ▾	<b>75.0</b>	0	25	0.000E+00 M	0.300

Figure 3-19: Dose-response data point table

6. Return to the *Dose-Response Curve* box (see Figure 3-19).

- ◆ Enter the *Assay Volume* added to all destination wells: **25.00 µL**
- ◆ Select the option **Automatic DMSO Backfill**.
- ◆ Select the timing for the backfill: **Last** (after compound transfer).

- ◆ Enter the maximum backfill volume: **75.nL**  
The maximum backfill volume enables you to limit the volume of DMSO if your assay cannot handle too much DMSO.  
The software calculates the backfill volume of DMSO and the final compound concentration for each data point, and then adds them to the dose-response data point table.
- ◆ Enter the maximum volume of DMSO to be filled by the Echo liquid handler: **60 nL**. Fill volumes greater than 60 nL must be backfilled with a bulk filler.

**Figure 3-20: Dose-Response Curve box**

The backfill offline volume gives you control over how much DMSO to fill with a bulk-filler. The bulk-filler can dispense larger volumes more quickly than the Echo liquid handler. Backfill volumes greater than or equal to the backfill offline volume (60 nL in this example) are highlighted in yellow in the data points table.

Please fill out the table below in its entirety.

#	Source Concentration	Transfer Volume (nL)	Backfill Volume (nL)	Assay Volume (µL)	Final Compound Concentration	Final %DMSO
1	5.000E-03...	75.0	0	25	1.496E-05 M	0.300
2	5.000E-03...	25.0	50	25	4.985E-06 M	0.300
3	5.000E-03...	7.5	67.5	25	1.496E-06 M	0.300
4	5.000E-03...	2.5	72.5	25	4.985E-07 M	0.300
5	5.000E-04...	75.0	0	25	1.496E-06 M	0.300
6	5.000E-04...	25.0	50	25	4.985E-07 M	0.300
7	5.000E-04...	7.5	67.5	25	1.496E-07 M	0.300

**Figure 3-21: Data point table showing offline volumes**

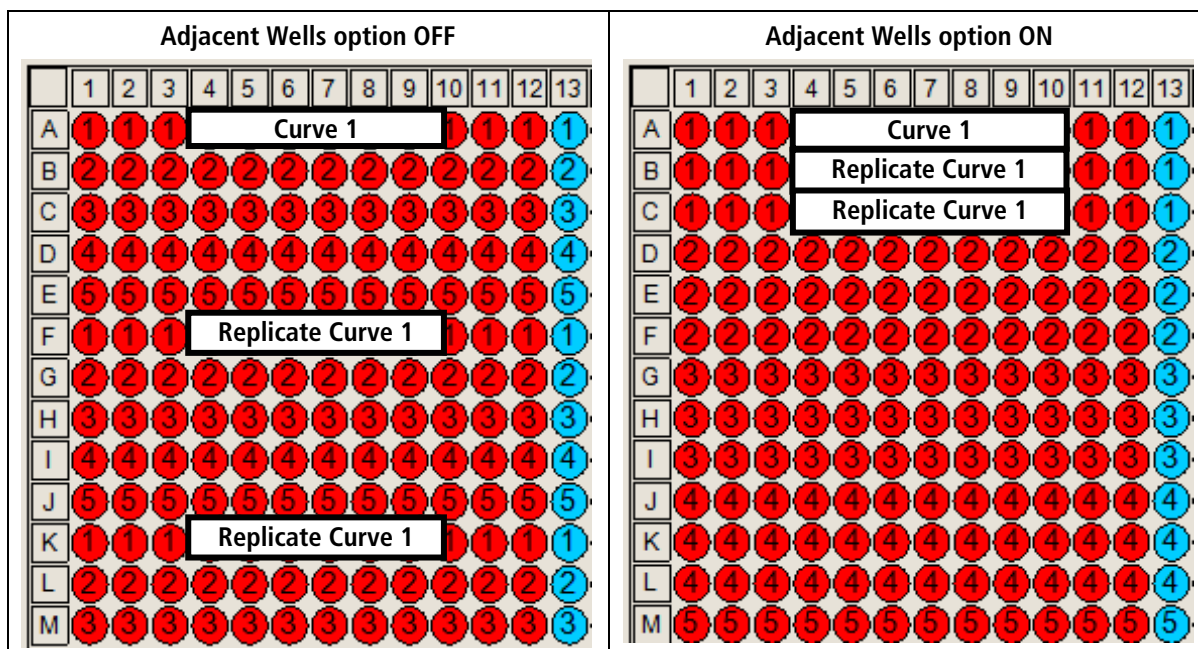
**Note:** The accuracy of offline back-filling depends on your bulk filler. If you need more accuracy than your bulk filler can provide (for example, 92.5 nL), then turn off the offline backfill option and the Echo liquid handler will perform the backfill for these wells.

7. Enter the following information for this protocol in the *Replicates* box:

- ◆ Enter the Number of Replicates: 3
- ◆ Select the **Same Plate** and **Adjacent Wells** options.
- ◆ Select the *Curve Fill Direction*: **Across**.
- ◆ Select the *Compound Fill Direction*: **Down**.

**Figure 3-22: Replicate information**

In the **Replicates** box, use the **Same Plate** option when you want replicate curves on the same plate; use **In Adjacent Wells** option if you want the replicates to follow each curve.



**Figure 3-23: Adjacent wells option**

**Note:** The software fits the entire curve and its replicates onto the same plate. If a curve and its replicates cannot fit on the plate, they are moved to the next plate.

Use the *Different Plates* option when you want the replicates on different plates.

The *Curve Fill* and *Compound Fill* options provide flexibility in specifying the order that the compounds, compound concentrations and controls are transferred to the destination plate.

Figure 3-24 shows the location of each compound and its dilutions for the Curve and Compound fill directions: **across, down**.

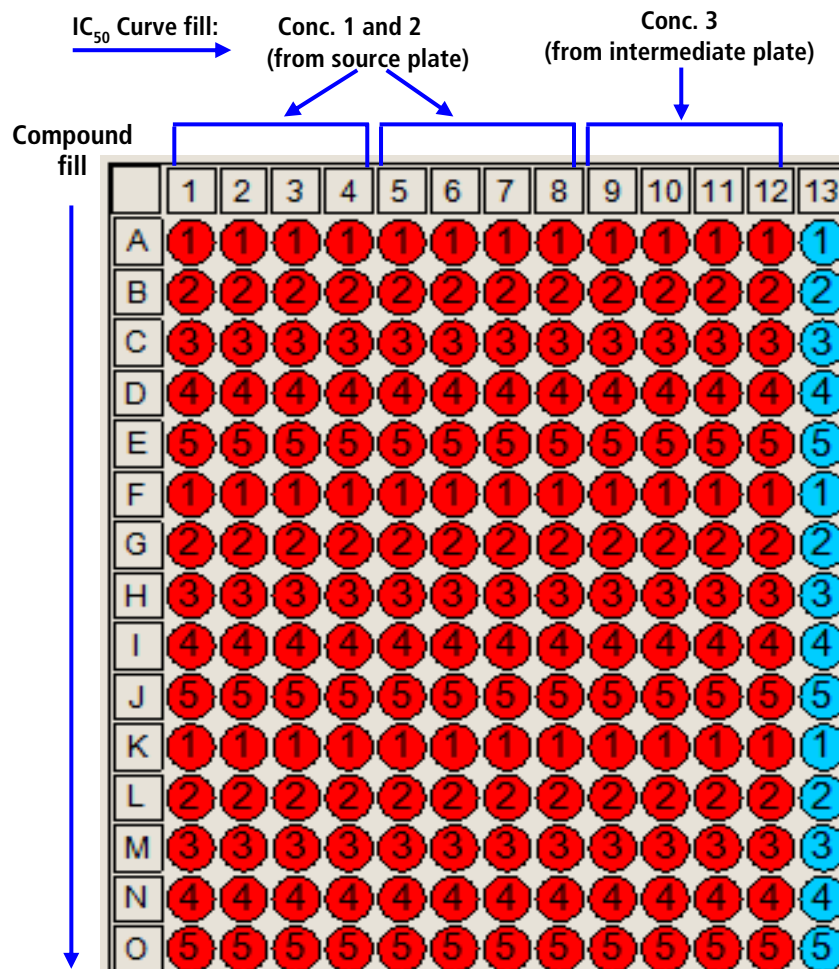
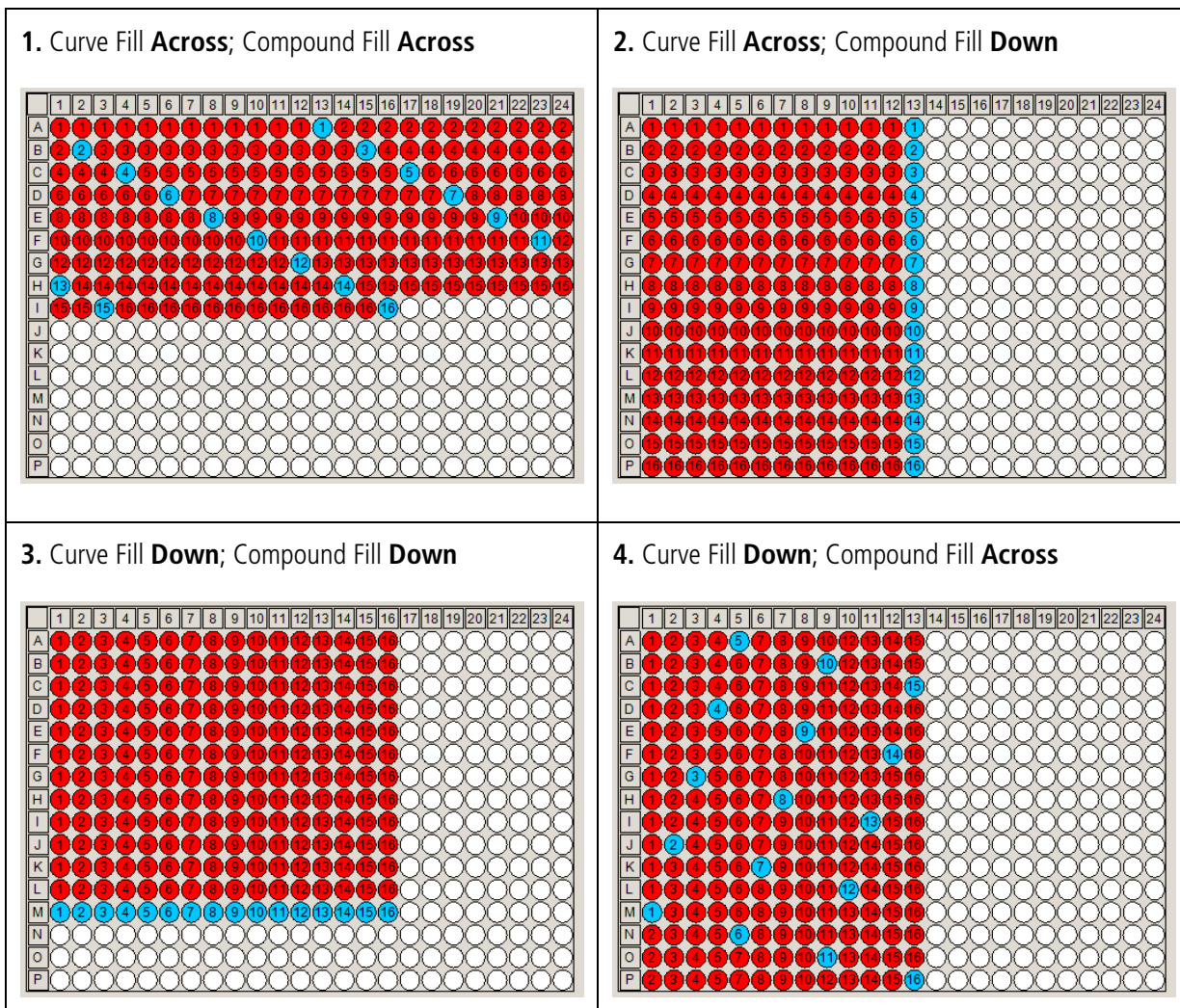


Figure 3-24: Destination plate design



Figure 3-25 shows additional configurations for curve and compound fill directions. The option you choose may depend on your testing requirements.



**Figure 3-25: Curve and compound fill options**

**Note:** The software fits all compound concentrations for a curve on the same plate. If a complete curve cannot fit on the plate, it is moved to the next plate. In configuration 3, the curve is filled down the 384-well plate. There is insufficient vertical room to fit more curves; therefore, only 12 curves can fit on the plate for this protocol. The other three configurations fit 16 curves.

When you have finished entering the curve data, it should look similar to the following figure:

Please fill out the table below in its entirety.

#	Source Concentration	Transfer Volume (nL)	Backfill Volume (nL)	Assay Volume (uL)	Final Compound Concentration	Final %DMSO
1	5.000E-03... ▾	75.0	0	25	1.496E-05 M	0.300
2	5.000E-03... ▾	25.0	50	25	4.985E-06 M	0.300
3	5.000E-03... ▾	7.5	67.5	25	1.496E-06 M	0.300
4	5.000E-03... ▾	2.5	72.5	25	4.985E-07 M	0.300
5	5.000E-04... ▾	75.0	0	25	1.496E-06 M	0.300
6	5.000E-04... ▾	25.0	50	25	4.985E-07 M	0.300
7	5.000E-04... ▾	7.5	67.5	25	1.496E-07 M	0.300
8	5.000E-04... ▾	2.5	72.5	25	4.985E-08 M	0.300
9	4.995E-06... ▾	75.0	0	25	1.494E-08 M	0.300
10	4.995E-06... ▾	25.0	50	25	4.980E-09 M	0.300
11	4.995E-06... ▾	7.5	67.5	25	1.494E-09 M	0.300
12	4.995E-06... ▾	2.5	72.5	25	4.980E-10 M	0.300
▶ 13	Positive ▾	75.0	0	25	0.000E+00 M	0.300

Figure 3-26: Dose-response data point window

- Click the **Next** button and enter the destination template name: **Destination Plate**.
- Click the **Finish** button to return to the *Plate Design* window.

The destination plate definition is complete.

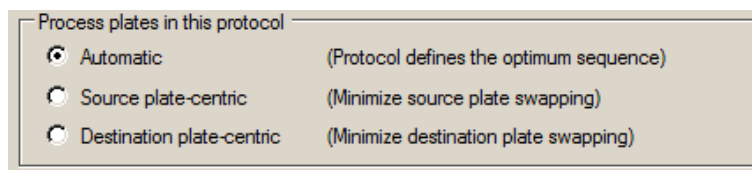
### 3.2.5 Specify order of transferring compound

The section *Process Plates in this protocol*/box is at the bottom of the *Plate Design* window. The plate processing order depends on your priorities. Select one of the following options:

**Automatic:** The software selects the most efficient order of plate handling. This is the default setting and may be fine for most protocols.

**Source plate-centric:** The software minimizes the frequency of swapping the source plate. This option is useful if you need to complete the processing of the source plate and return it to storage.

**Destination plate-centric:** The software minimizes the frequency of changing the destination plate. This option is best if you need to complete the processing of the destination plate and put it in environmentally-controlled plate storage.



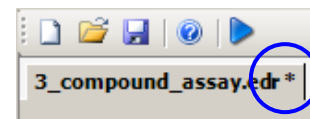
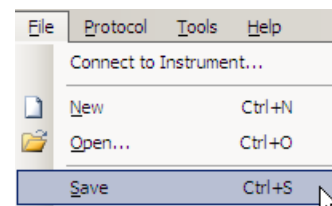
**Figure 3-27: Process plate options**

**Hint:** You can save processing time by turning on the **Survey History** function. This function saves survey data for the source plate and reuses it the next time that the source plate is returned to the Echo liquid handler. For more information, go to "*Survey History*", page. 80.

For this sample protocol, use the **Automatic** option.

### 3.2.6 Save the protocol.

1. Open the *File* menu in the *Toolbar*.
2. Select **Save**.
3. Enter a file name in the prompt box. The protocol is saved with the file extension ".edr" in C:\Labcyte\Echo\Protocols, the default directory. You can save the file to a different directory.
4. When you edit the template, an asterisk appears next to the file name. This is a reminder that the file has information that has not been saved.

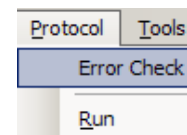


### 3.2.7 Check for Errors

The Echo Dose-Response software contains many checks and prompts during the creation of each plate template. However, it is useful to do a check of the entire protocol before running it. This program looks for inconsistent information.

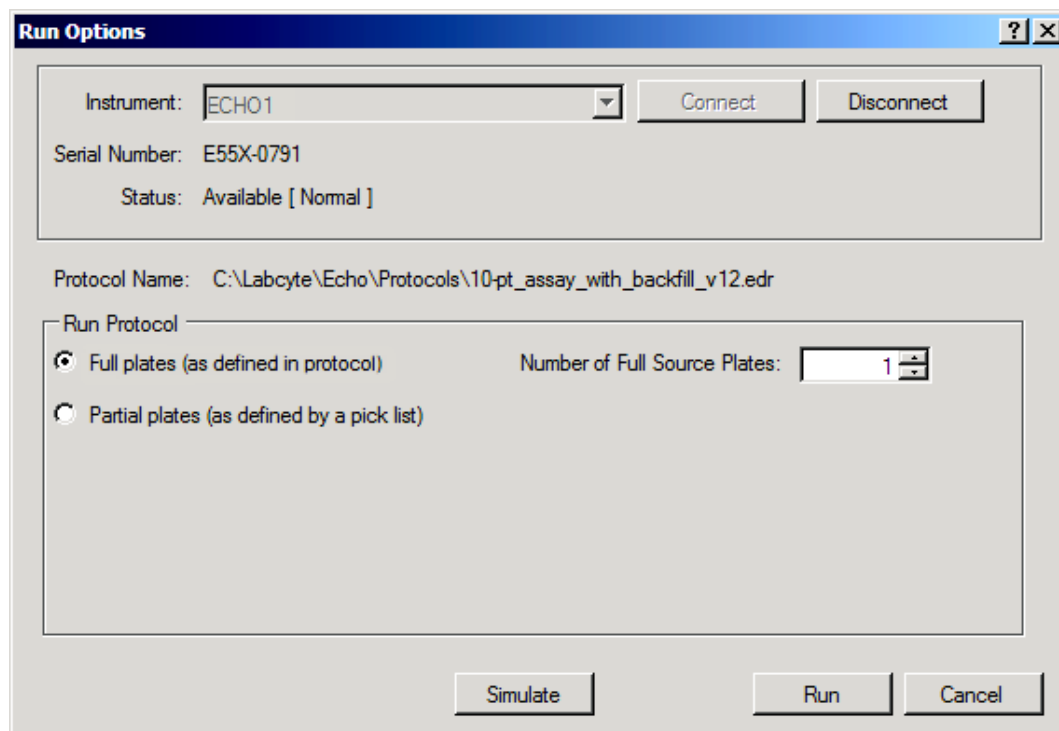
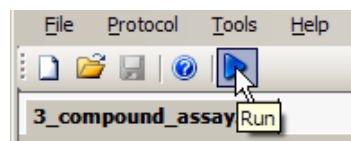
- Select **Error Check** from the *Protocol* menu.

The transfer protocol is done.



### 3.3 Select a Protocol Run

The *Run Options window* opens when you click the **Run** icon in the Echo Dose-Response toolbar. This window gives you two run functions: **Simulate** and **Run**.



**Figure 3-28: Run Options window**

**Simulate:** The *Simulate* option opens a mock run window to allow you to simulate the protocol on the computer before performing the actual transfer. This option is available when you are working offline or when connected to the Echo liquid handler. Go to "Run a Simulation " for more information and procedures.

**Run:** The *Run* option opens the run status window to run the transfer protocol. This option is available only when you are connected to the Echo liquid handler. The prompt box contains a **Connect** button to communicate with the Echo liquid handler. Go to "Run the Protocol" on page 44 for more information and procedures.

---

The *Run Options window* also contains the following options:

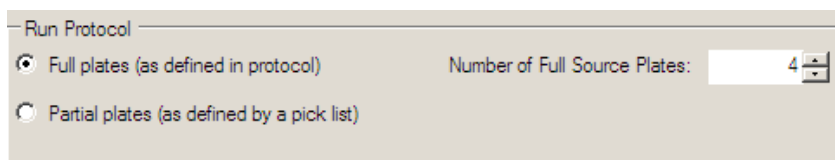
**Connect/Disconnect:** This option enables you to connect to the Echo liquid handler directly from the *Run Options window* instead of opening the *Connect* window. After you click **Connect**, the software displays the instrument serial number and availability (normal or busy).

---

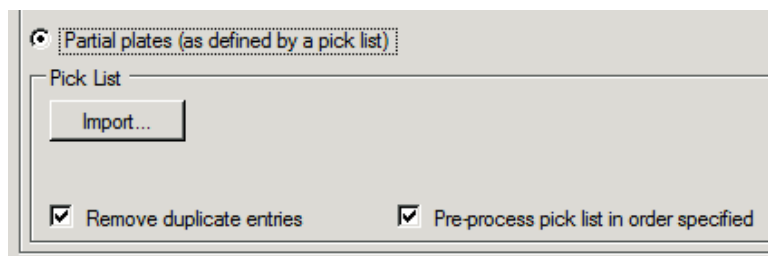
**Note:** If you receive a connection error “Host refused connection. Verify address and port number...”, then the Echo liquid handler is turned off. Turn on the Echo liquid handler, wait for the green ready light to appear, and then click **Connect** again.

---

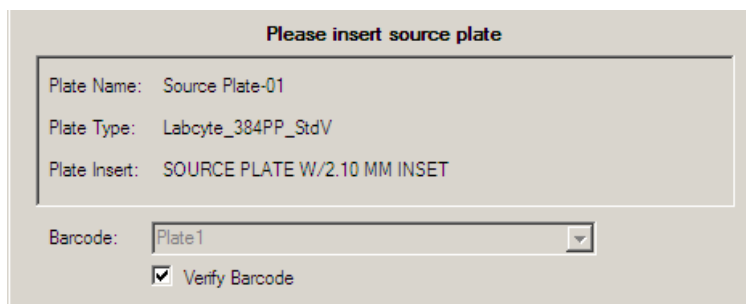
**Full plates:** This option allows you to run *full plates* as defined by the protocol you created. The setting, **Number of Full Source Plates**, allows you to process multiple source plates in a single run. See protocol example 2 in the Appendix.



**Partial plates:** This option allows you to run *partial plates* from an imported pick list. When you select the *Partial plates* option, an **Import** button is displayed to choose a CSV-formatted list of plates and wells to process. You also have the following optional settings:



- ◆ **Remove Duplicate Entries.** The software ignores duplicate wells that contain the same compound (removes duplicates from the processing list).
- ◆ **Pre-process pick list in the order specified.** The software pre-processes the pick list to determine the placement of all compounds *in advance* of the run. During the run, the software prompts you for the specific source plate needed.



## 3.4 Run a Simulation

### What is a simulation?

The simulation program is a computer-generated execution of the transfer protocol. When you click **Simulate** in the *Run Options window*, the software displays a mock run window that shows the source and destination plate maps, protocol information and run status, and animation of the transfer.

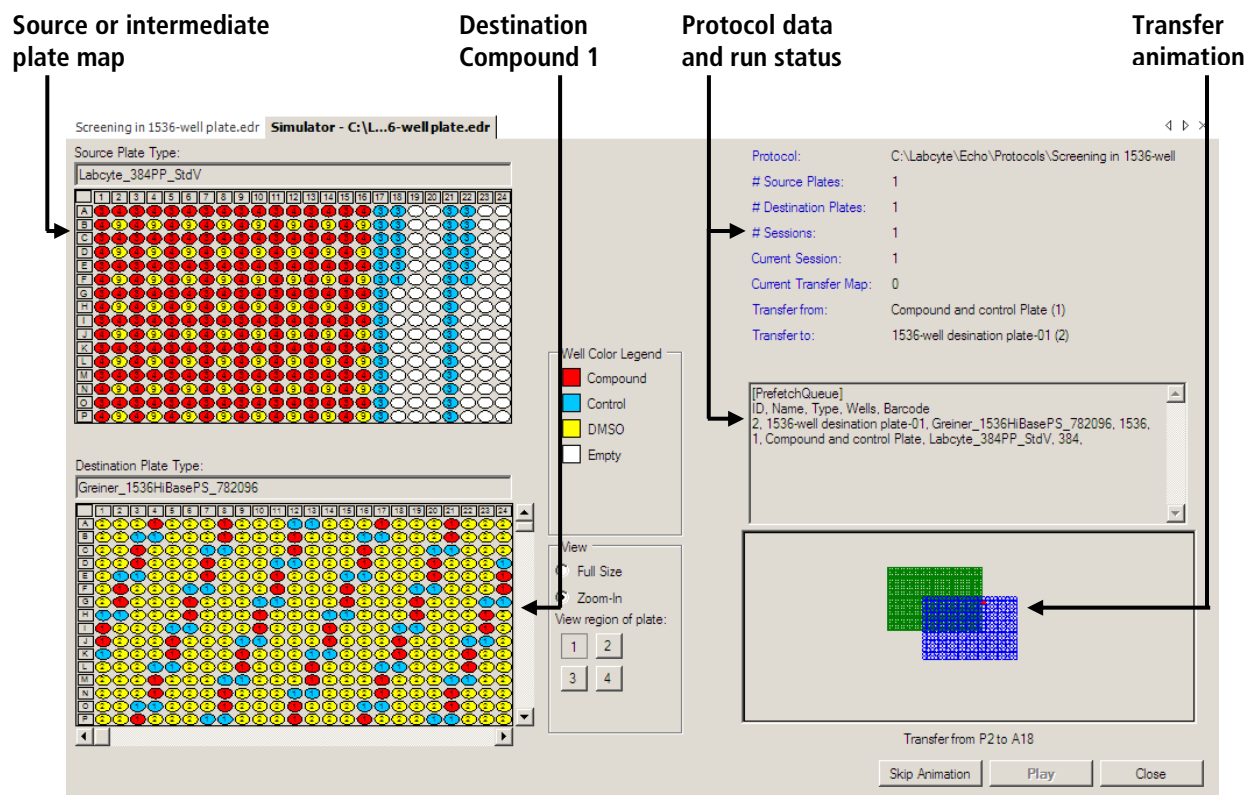



Figure 3-29: Run simulation screen

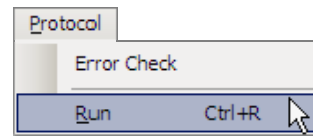
The simulation is very useful for testing a newly created protocol and should be run before committing time and material to the actual transfer run. Because the simulation runs very quickly, you can test several different plate configurations.

The simulation can also be used to assemble the plates needed for the transfer protocol. The protocol data in the upper right area of the screen displays the number of source, intermediate (if used), and destination plates that are needed to run the protocol.

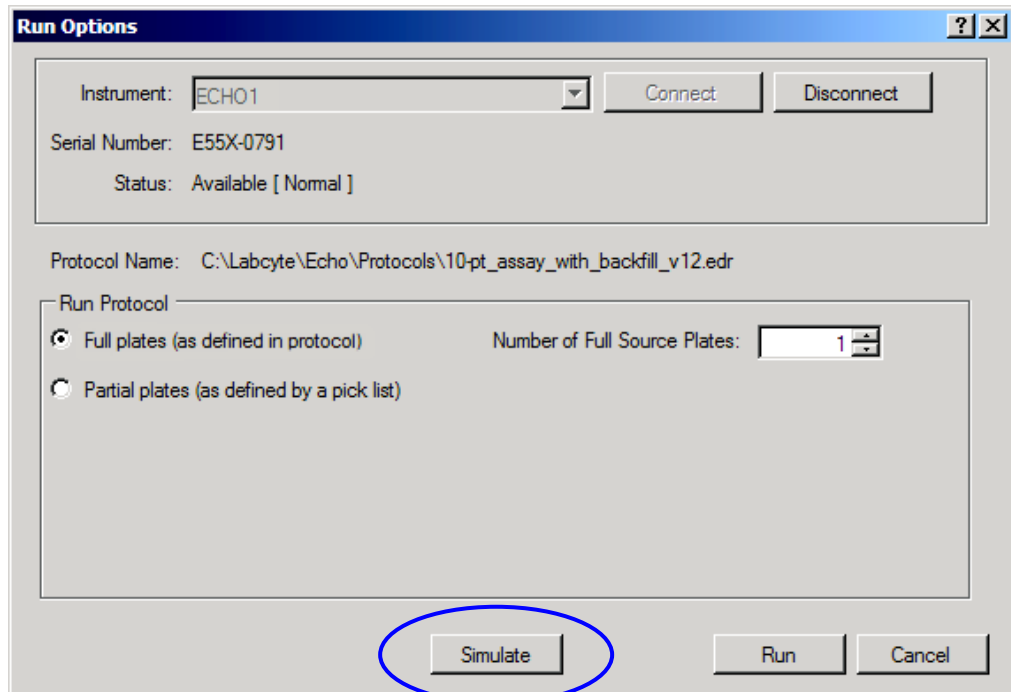
For an in-depth discussion on how the simulation works, go to page 70.

**To run a protocol simulation:**

1. Click the **Run** icon  or select **Run** from the *Protocol* menu. If you have not saved your protocol, you will be prompted to save it now.



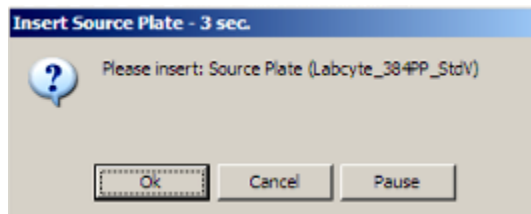
2. Enter the **Number Of Full Source Plates** that will be used in this run.
3. Click the **Simulate** button in the *Run Options window*.



**Figure 3-30: Run Options window, offline**

4. Click the **Play** button (lower right corner of the window) to start the simulation run. If the protocol is complex, or contains several plates, the software will require extra time to load the information.

To simulate a transfer run, the software will display prompts to “insert” the source, intermediate, and destination plates into the Echo liquid handler. You can respond to these prompts or allow the software to respond to the next logical step (after a 5-second delay).



**Figure 3-31: Plate prompt during transfer simulation**

During the simulation you can click **Skip Animation** or **Cancel**.

- Click **OK** when the simulation is complete. The following figures show a simulation in progress and completed.

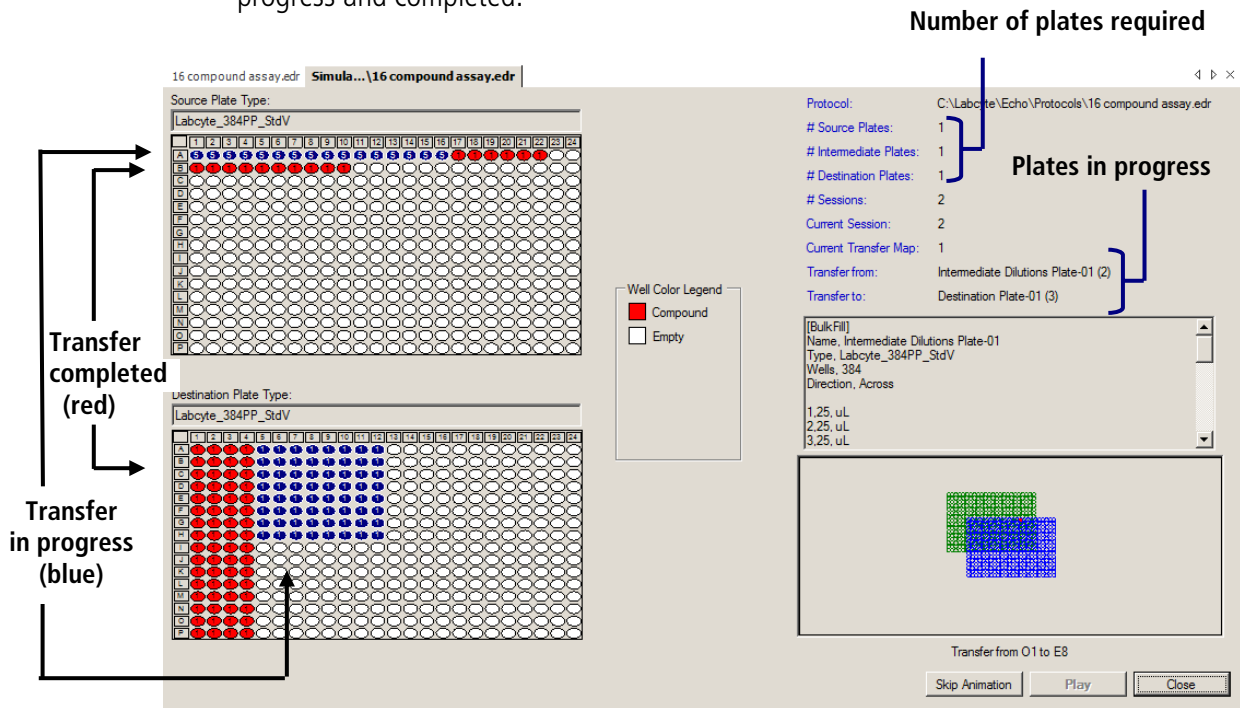


Figure 3-32: Simulation in progress

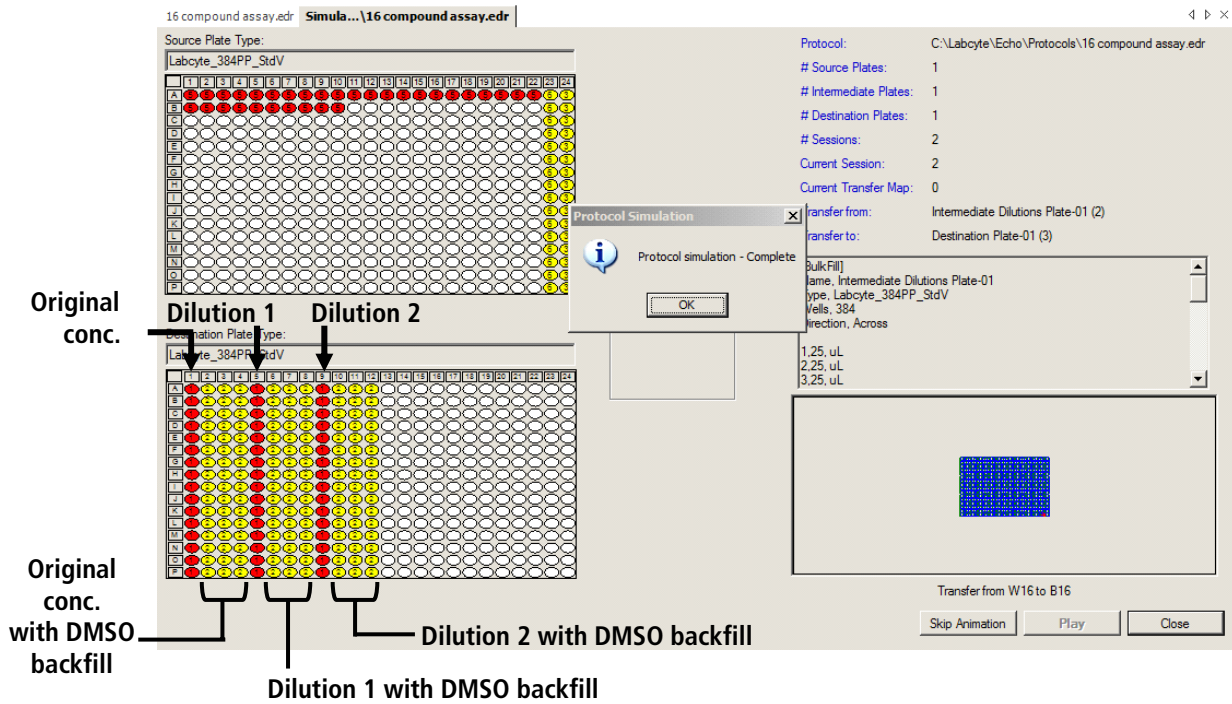


Figure 3-33: Simulation completed



---

## 3.5 *Set up Plates*

Use the design of the plate templates and the number of plates specified in the simulation to set up the plates for your compound transfer.

1. Prepare an Echo-qualified source plate with the compounds you will be transferring. Manually pipet Compound 1 in well A1, Compound 2 in well B1, and so on, as shown in the source plate template.



**Figure 3-34: Preparing source plate**

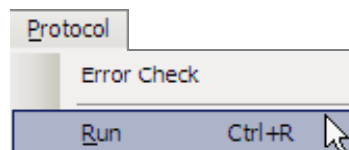
2. Prepare an intermediate plate. Manually pipet compound dilutions and DMSO into the assigned wells.
3. Collect the number of destination plates that are specified in the simulation to receive the final compound concentrations.

When your plates are ready for the liquid transfer, continue to the next section to run the transfer protocol.

### 3.6 Run the Protocol

After you have created a transfer protocol and tested it in the software simulator, you are ready to run the protocol on an Echo liquid handler system:

1. Click the **Run** icon  or select **Run** from the *Protocol* menu.



2. Select **Full plates** or **Partial plates**:

- ◆ **Full plates:** Run the source plate exactly as it was defined in the Source Plate Designer. Specify how many compound source plates will be used in this run.
- ◆ **Partial plates:** Import and select a pick list. Determine if you want to use the options: **Remove Duplicate Entries** and **Pre-process pick list in order specified**.

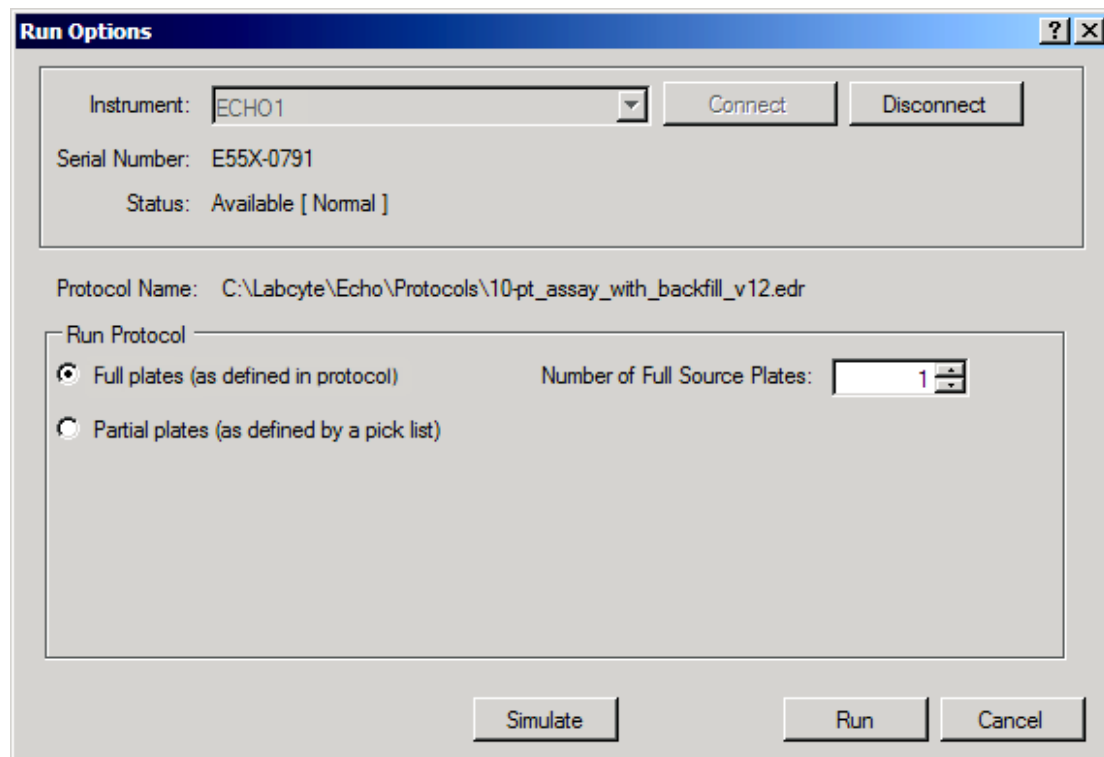


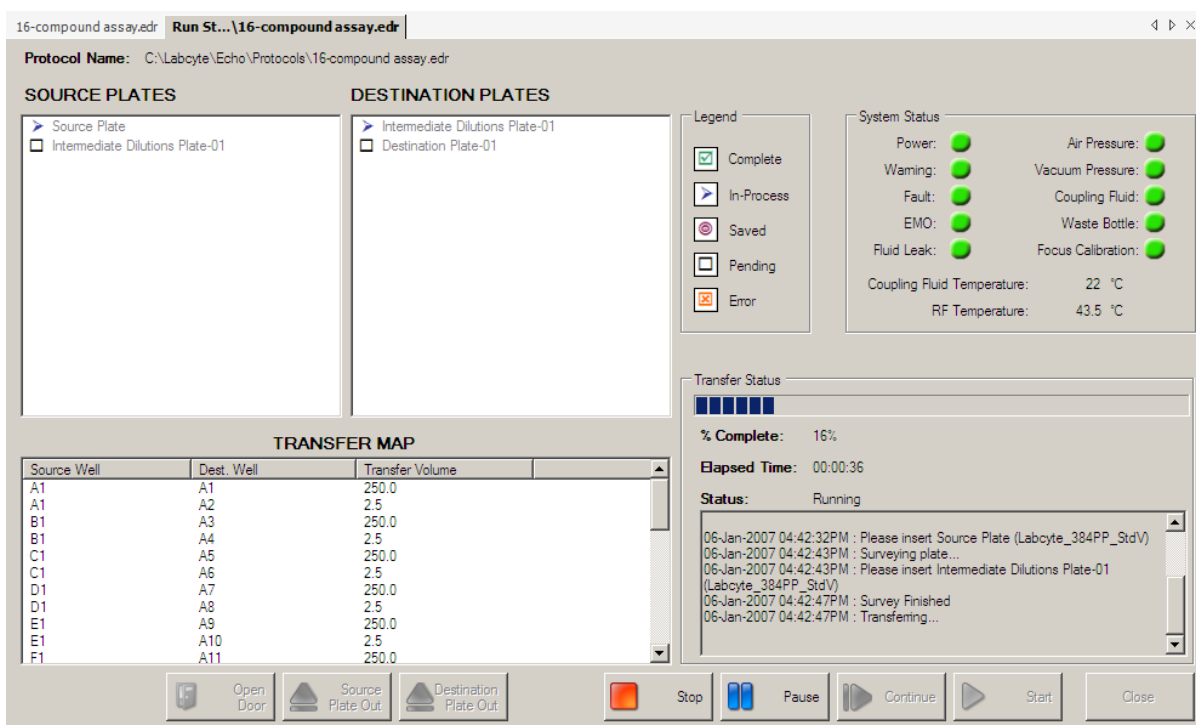
Figure 3-35: Run Options window, connected to instrument

3. Click **Run** in the *Run Options window* to open the *Run Status window*.


**Note:** If the **Run** button is inactive (grayed out), you are working offline. Select an instrument and click **Connect**.

The *Run Status window* is a dynamic window that provides control buttons to run the protocol. It also contains various status windows to show the progress of the compound transfer, the state of the Echo liquid handler, and the final well positions and volume transferred.

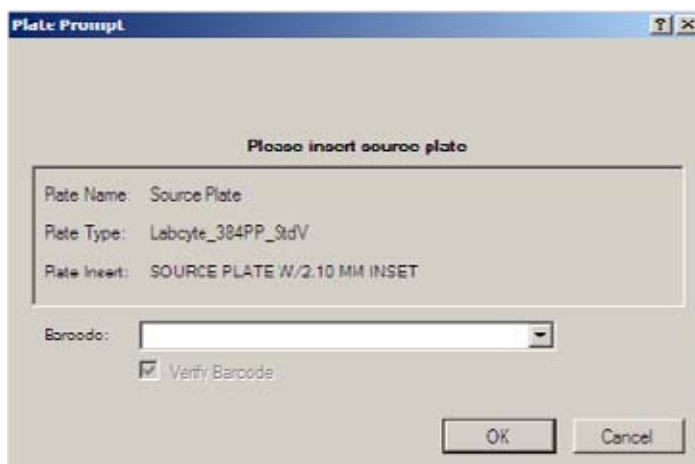
Figure 3-36 shows a run in progress.



**Figure 3-36: Transfer protocol in progress**

4. Click the **Start** button  to begin the sample protocol. The software begins by displaying prompts to remove plates currently in the Echo liquid handler.
5. Click **OK** in the plate prompts after you remove any existing plates from the Echo liquid handler.

The first plate prompt for the sample protocol is to insert the source plate.



**Figure 3-37: Source plate prompt during transfer run**

The prompt box displays the plate template name, plate type, plate insert, and a field for the **barcode** or **plate ID**.

When you are using multiple source, intermediate, or destination plates, the barcode or plate ID field provides an additional check, **Verify Barcode**, to identify the correct plate. The software will compare the barcode you enter to the plate that is inserted in the Echo liquid handler (and scanned by the barcode reader). If the barcode doesn't match, or if you enter the same barcode for two different plates, then the software will display an error message. If you use plate IDs, the software will check for inconsistencies (for example, using the same plate ID for two different plates) and display an error message.

---

**Note:** If you are running a pick list and you selected **Pre-process pick list in the order specified** in the *Run Options* window, the software displays the source plate barcode or ID that should be inserted during the run. In this situation, the Barcode field cannot be edited.

---

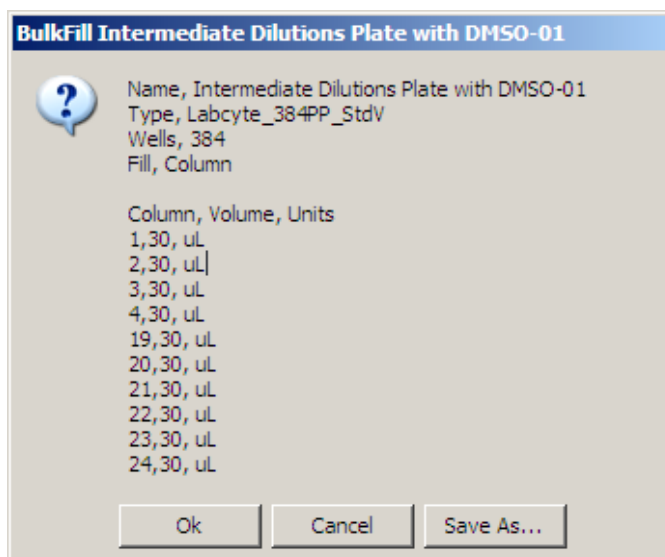
You can disable barcode verification by deselecting the **Verify Barcode** option after you have entered a barcode or plate ID in the *Barcode* field.

You can also skip the *Barcode* field entirely and click **OK**. This sample protocol uses only one source, intermediate, and destination plate; therefore, you will skip the *Barcode* field.

6. Follow the directions in each plate prompt and click **OK**.

The *Run Status* window will continuously update as the transfer process continues.

When the run reaches the bulk-fill step, the software will display the *Bulk Fill* prompt box.



**Figure 3-38: Bulk-fill status during transfer run**

You can use this information to pipet DMSO into the bulk-fill wells of the intermediate plate.

If you prefer to save it to file (for example, to print it), use the following steps:

- a. Click the **Save As** button to open the *Save* dialog box.
- b. Browse to the folder that will store the file. The default folder is C:\Labcyte\Echo\Protocols.
- c. Name the file, select **csv** as the file type, and click **Save**.
- d. Open the csv file in a spreadsheet program.

	A	B	C	D	E	F
1	Name	Intermediate Dilutions Plate with DMSO-01				
2	Type	Labcyte_384PP_StdV				
3	Wells	384				
4	Fluid	DMSO				
5	Fill	Column				
6						
7	Column	Volume	Units			
8	1	30	uL			
9	2	30	uL			
10	3	30	uL			
11	4	30	uL			

**Figure 3-39: Bulk-fill data in a spreadsheet**

At any time during the protocol run, you can pause or stop it. When the protocol run is done, the progress bar will show 100% complete and the **Close** button will become active.

### 3.7 Manage Transfer Data

At the end of the transfer run, the Echo Dose-Response software builds the transfer reports and stores them in dated directories in the following location:

C:\Labcyte\Echo\Reports\Labcyte Echo Dose-Response\

Name	Size	Type	Date Modified
1167789741--Intermediate Dilutions Plate-01.BulkFill	1 KB	BULKFILL File	1/2/2007 6:54 PM
E55X-0790_Print_1167792868.csv	2 KB	Microsoft Office Excel ...	1/2/2007 6:57 PM
E55X-0790_Print_1167792868.xml	10 KB	XML Document	1/2/2007 6:57 PM
E55X-0790_Print_1167792879.csv	8 KB	Microsoft Office Excel ...	1/2/2007 6:57 PM
E55X-0790_Print_1167792879.xml	89 KB	XML Document	1/2/2007 6:57 PM
E55X-0790_Survey_Intermediate Dilutions Plate-010.csv	16 KB	Microsoft Office Excel ...	1/2/2007 6:57 PM
E55X-0790_Survey_Intermediate Dilutions Plate-010.xml	144 KB	XML Document	1/2/2007 6:57 PM
E55X-0790_Survey_Source Plate0.csv	1 KB	Microsoft Office Excel ...	1/2/2007 6:57 PM

Figure 3-40: Transfer report directory

From this directory location you can retrieve the following reports:

	A	B	C	D	E	F	G	H
1	Run ID	1168130535						
2	Run Date/Time	2007-Jan-06 04:42:15PM						
3	Application Name	Labcyte Echo Dose-Response						
4	Application Version	1.0.0						
5	Protocol Name	16-compound assay.edr						
6	User Name	jsmith						
7								
8	[DETAILS]							
9	Source Plate Name	Source Plate Barcode	Source Plate Type	Source Well	Survey Fluid Height	Survey Fluid Volume	% DMSO	Survey Status
10	Source Plate	Labcyte_384PP_StdV	A1	2.364	36.629	85		
11	Source Plate							
12	Source Plate	1	Run ID	1.2E+09				
13	Source Plate	2	Run Date/Time	2007-Jan-06 04:42:15PM				
14	Source Plate	3	Application Name	Labcyte Echo Dose-Response				
15	Source Plate	4	Application Version	1.0.0				
16	Source Plate	5	Protocol Name	16-compound assay.edr				
17	Source Plate	6	User Name	jsmith				
18	Source Plate	7						
19	Source Plate	8	[DETAILS]					
20	Source Plate							
21	Source Plate			Source Plate Barcode	Source Well	Survey Fluid Height	Survey Fluid Volume	% DMSO
22	Source Plate							Survey Status
23	Source Plate	9	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A1	2.364	36.629
24	Source Plate	10	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A2	2.364	36.629
25	Source Plate	11	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A3	2.364	36.629
26	Source Plate	12	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A4	2.364	36.629
27	Instrument Name	13	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A5	2.364	36.629
28	Instrument Model	14	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A6	2.364	36.629
		15	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A7	2.364	36.629
		16	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV	A8	2.364	36.629
		17	Source Plate Name	Intermediate Dilutions Plate-01	Labcyte_384PP_StdV			

Figure 3-41: Intermediate and source plate survey reports in csv format



## 4 Software Reference

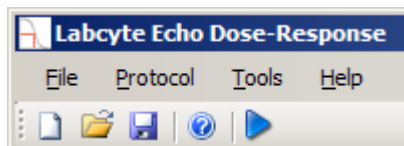
This chapter contains descriptions of all the Echo Dose-Response software screens. This information and procedures for each screen are available in the Echo Dose-Response online Help.

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## 4.1 *Toolbar*

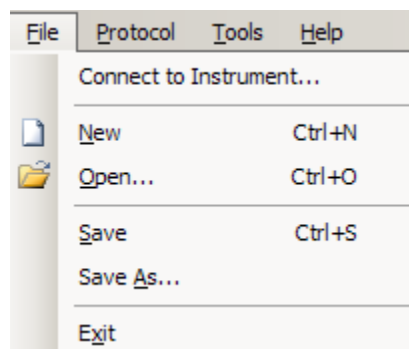
The *Toolbar* contains the *File*, *Tools*, and *Help* menus, as well as individual icons for frequently-used functions.



From the *Toolbar*, you can perform the following functions:

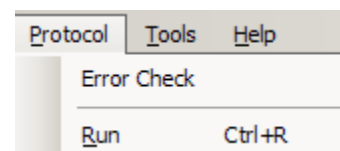
### File menu

- ◆ **Connect to instrument:** Select an Echo liquid handler to connect to the software
- ◆ **New:** Create a new transfer protocol
- ◆ **Open:** Open an existing transfer protocol
- ◆ **Save:**\* Saves the current transfer protocol
- ◆ **Save As:**\* Copies the current transfer protocol to a different file name
- ◆ **Exit:** Closes the Echo Dose-Response software.



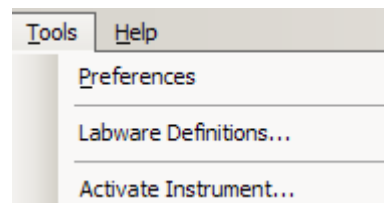
### Protocol menu\*

- ◆ **Error Check:** Checks the protocol for inconsistent information
- ◆ **Run:** Executes the transfer protocol that is displayed.



### Tools menu

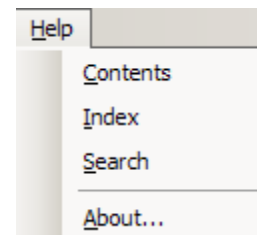
- ◆ **Preferences:** Set survey, output, and report options for all protocols.
- ◆ **Labware definitions:** Manage existing labware definitions or add new definitions.
- ◆ **Activate the instrument:** Enter software license key to use the Echo Dose-Response software on each Echo liquid handler.



\* Available only when a protocol is open.

## Help menu

- ◆ **Contents:** View the Help table of contents.
- ◆ **Index:** Search Help through available key words.
- ◆ **Search:** Search Help through words or phrases.
- ◆ **About:** Display the version number of the Echo Dose-Response software.



## 4.2 Plate Design

The *Plate Design* window contains the protocol name, description, and plate templates.

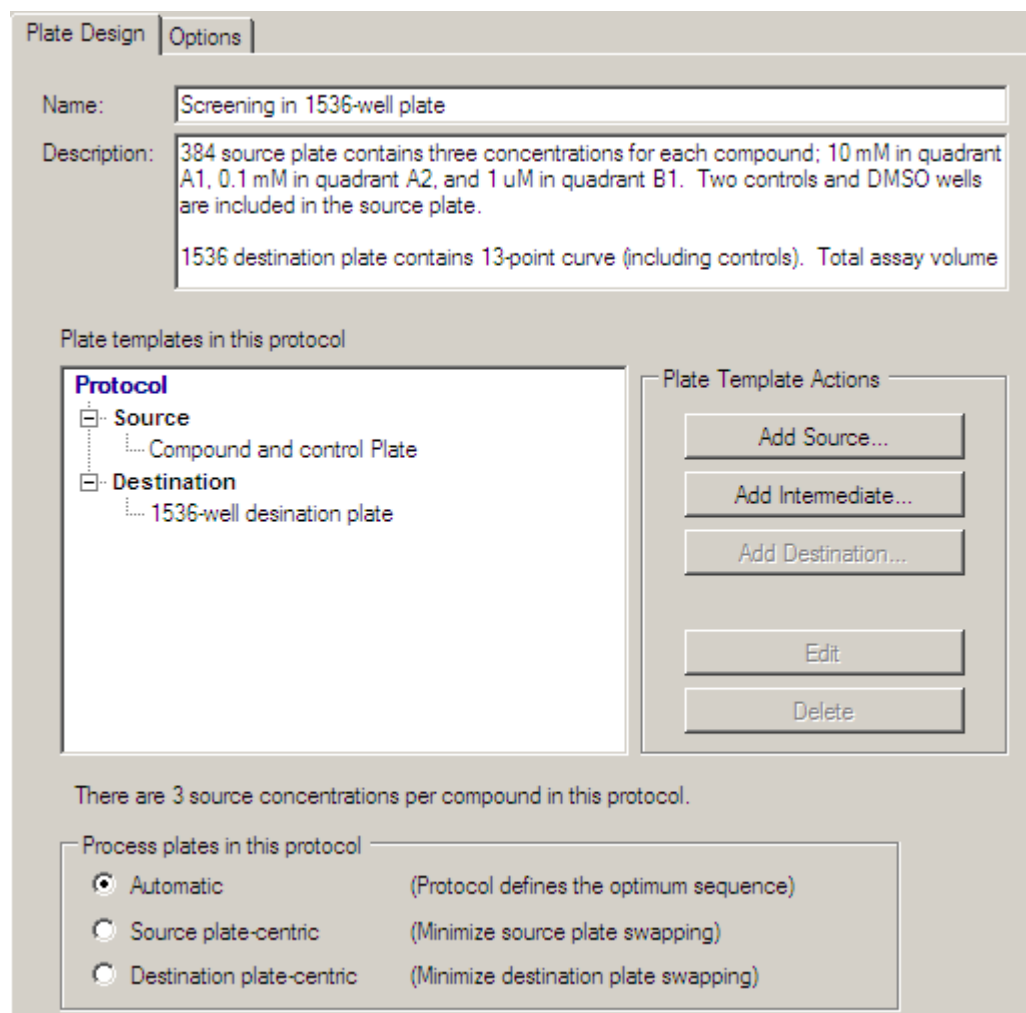


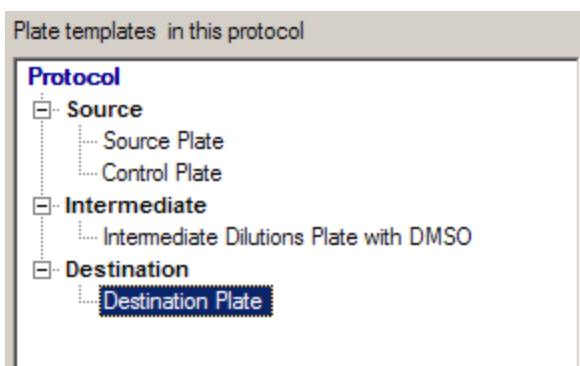
Figure 4-1: Plate Design Window

---

**Name:** Name of the template for the transfer protocol. This is a user-assigned name that is different than the filename.

**Description:** Additional text that describes the template, such as the list compounds that are being transferred, concentrations, number of points on the dose-response curve, and controls and backfill, if they are used.

**Plate templates in this protocol:** List of plate designs (templates) in a *directory tree* that are used in this protocol. Double-click the template in the directory tree to open it.



**Figure 4-2: Plate Template Tree**

### Plate Template Actions

**Add Source:** Opens the Source Plate Designer wizard to create the source plate template and add it to the Protocol tree.

**Add Intermediate:** Opens the Intermediate Plate Designer wizard to create the source plate template and add it to the protocol tree.

**Add Destination:** Opens the Destination Plate Designer wizard to create the source plate template and add it to the protocol tree.

**Edit:** Opens the selected plate template in the protocol tree for editing

**Delete:** Deletes the selected plate template in the protocol tree.

### Plate processing order

**Automatic:** most efficient order of compound transfer. Default setting.

**Source plate-centric:** minimizes frequency of changing source plate. This option is useful if you need to complete the processing of the source plate and return it to storage.

**Destination plate-centric:** minimizes frequency of changing destination plate. This option is best if you need to complete the processing of the destination plate and put it in environmentally controlled plate storage.

## 4.2.1 Source Plate Designer

The **Add Source** button begins the *Source Plate Designer* wizard in a new window. This wizard helps you prepare a template for transferring compound from the source plate to one or more destination plates.

**Step 1: Design the Source Plate Template:** Defines the source plate type and assigns wells to compound, control, or DMSO. See "*Step 1 Fields and Buttons*" below.

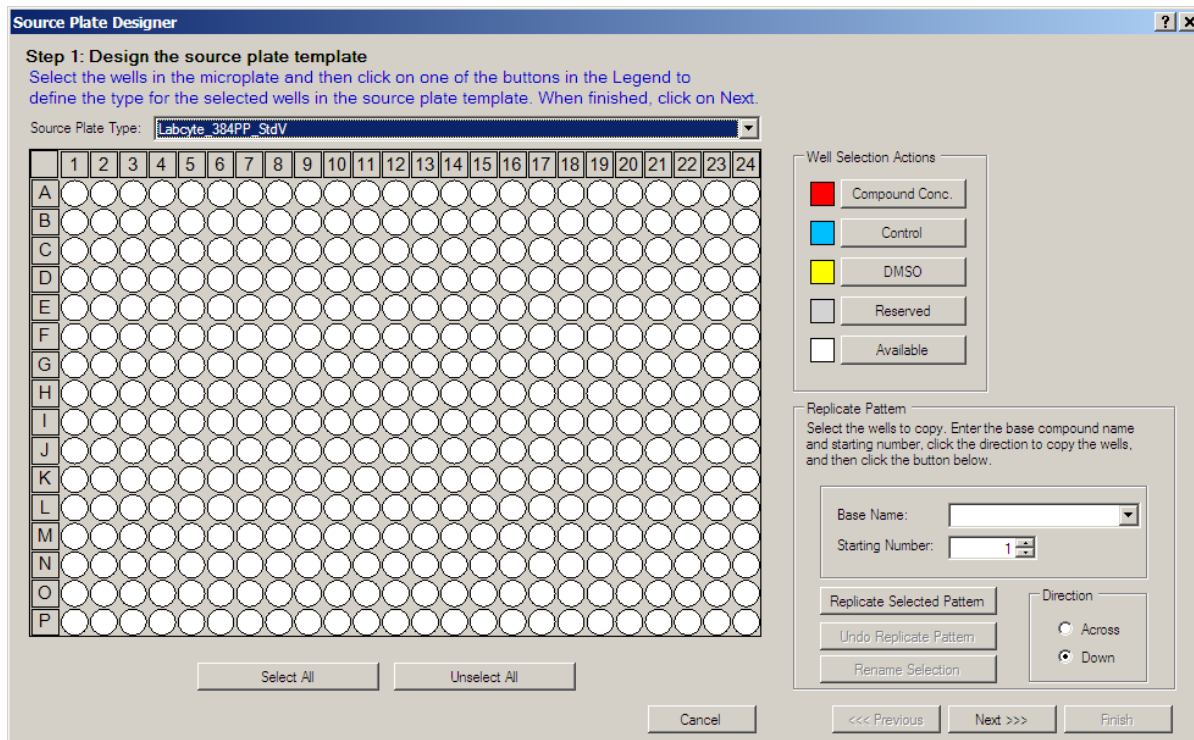







Figure 4-3: Source Plate Designer

### *Step 1 Fields and Buttons:*

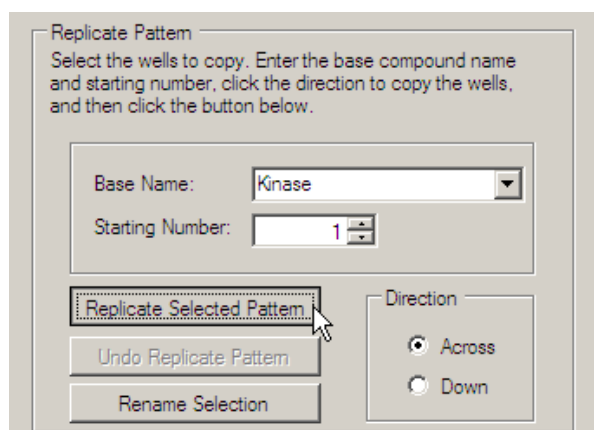
**Source Plate Type:** Drop-down menu of available source plates. Source plate must be an Echo-qualified plate.

**Plate map of the selected source plate:** well positions that can be clicked and assigned to the components in the *Well Selection Action* box:

**Well Selection Action:** Components that can be assigned to the selected well positions:

 <input type="button" value="Compound Conc."/>	Assigns one or more compounds or compound concentration. Opens a prompt box to enter the compound concentration.
 <input type="button" value="Control"/>	Assigns negative, positive, or other control compound.
 <input type="button" value="DMSO"/>	Assigns wells to hold DMSO for backfilling assay wells in the destination plate.
 <input type="button" value="Reserved"/>	Prevents liquid transfer to or from the selected wells.
 <input type="button" value="Available"/>	By default, all well positions are available. Use this button to deselect well positions that have been previously selected.
<input type="button" value="Select All"/>	Selects all wells in the plate template for quick well assignment. Same action as clicking the corner cell above well <b>A</b> .
<input type="button" value="Unselect All"/>	Unselects all wells in the plate template that were previously selected.

**Replicate Pattern box:** Replicates a selected well or pattern of wells to all available wells in the source plate.



**Figure 4-4: Replicate Pattern box**

**Base Name:** Applies a base name to selected and replicated compound wells. Also replaces an existing name if the Rename Selection function is used.

**Starting Number:** Begins the numbering sequence. After the selected wells are replicated and numbered, this field displays the next available replicate number.

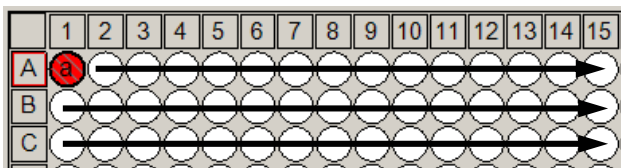
**Replicate Selected Pattern:** Replicates and numbers selected wells (pattern) in the direction that is specified (across or down). Applies base name and sequentially numbers all compound wells.

**Undo Replicate Pattern:** Reverses the last replicate function.

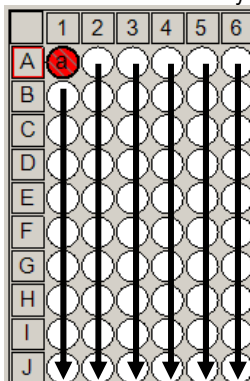
**Rename Selection:** Renames selected compound wells with the text in the Base Name field. Also renumbers the wells from the Starting Number. Does not affect control or DMSO wells.

**Direction box:** Specifies the direction to replicate the wells. All selected components (compound, control, DMSO) are replicated, but only the compound wells are numbered.

**Across:** Replicates the selected well horizontally in all available wells in the plate.



**Down:** Replicates the selected well vertically in all available wells on the plate.



**Step 2: Choose a name for the source plate template:** Provides a name for the source plate template.

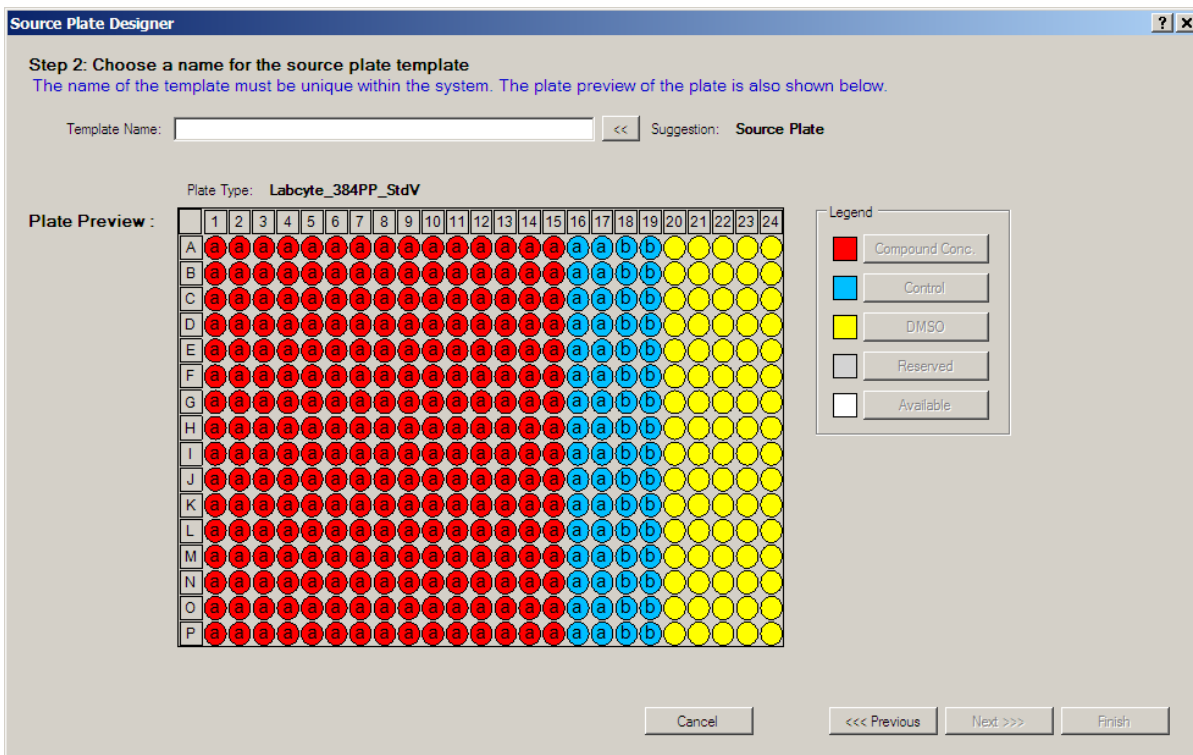


Figure 4-5: Source Plate Designer, step 2

## 4.2.2 Intermediate Plate Designer

The **Add Intermediate** button begins the *Intermediate Plate Designer* wizard in a new window. This wizard help you prepare a template for additional compound concentrations.

**Step 1: Select wells to reserve or bulk-fill with DMSO only:** Defines the intermediate plate type. Assigns wells to DMSO or blocks wells from compound transfer. See "*Step 1 Fields and Buttons*" below.

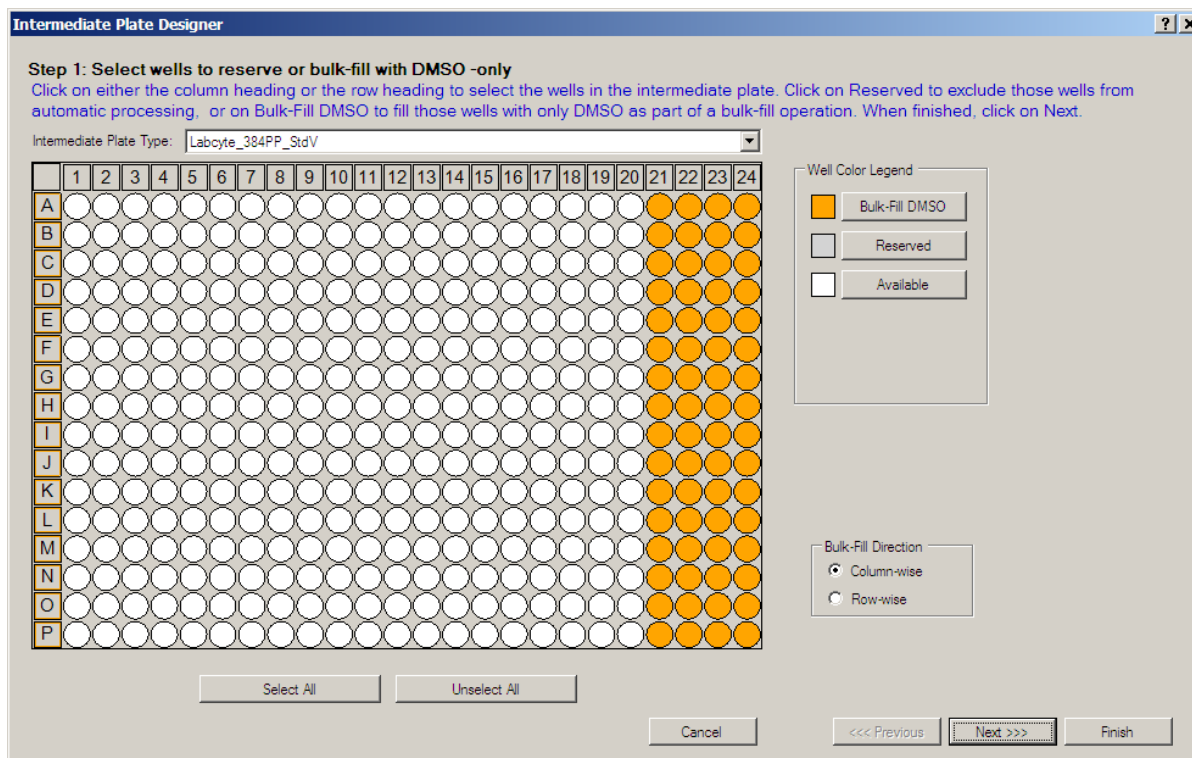


Figure 4-6: Intermediate Plate Designer



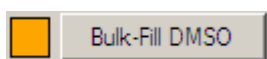
---

### **Step 1 Fields and Buttons:**

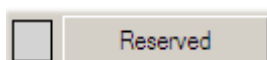
**Source Plate Type:** Drop-down menu of available source plates

**Plate map of the selected intermediate plate:** well positions that can be clicked and assigned to the components in the Well Selection Action box:

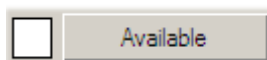
**Well Selection Action:** Components that can be assigned to the selected well positions (see below)



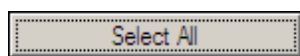
Assigns wells to hold DMSO for bulk filling compound wells in the intermediate plate or assay wells in the destination plate.



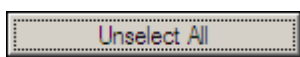
Prevents liquid transfer to or from selected wells.



By default, all well positions are available. Use this button to deselect well positions that have been previously selected.



Selects all wells in the plate template for quick well assignment. Same action as clicking the corner cell above well **A**.



Unselects all wells in the plate template that were previously selected.

**Bulk Fill Direction:** specifies the direction that the assay wells are to be filled by a bulk filler.

---

**Note:** The selected wells are used as reservoirs for DMSO only. During plate preparation (or during the protocol run when the bulk-fill screen is displayed) the selected wells are filled with DMSO, either manually or with a bulk filler. The Echo system uses the DMSO in these wells to backfill the compound wells in the assay plate.

---

**Step 2: Build Intermediate Plate:** Specifies the number of intermediate transfers and calculates the compound concentrations.  
See "*Step 2 Fields*".

Intermediate Plate Designer

**Step 2: Build Intermediate Plate**  
Select the number of intermediate concentrations, and fill out the table below.  
Choose the transfer volume and select the bulk fill volume per new concentration. Click on Next when finished.

Intermediate transfers

How many intermediate concentrations would you like? 1

Source Concentration	Transfer Volume	Bulk-Fill Volume	New Concentration
0.005 M	150.0 nL	30.00 μL	2.488E-05 M

Cancel <<< Previous Next >>> Finish

Figure 4-7: Source Plate Designer, Step 2

***Step 2 Fields:***

**How many intermediate concentrations would you like?** Number of intermediate concentration fields to be listed in the concentration table.

**Source Concentration:** Drop-down list of source concentrations previously defined in the source plate template.

**Transfer Volume:** Volume of source compound (in nanoliters) to be transferred from the source plate.

**Bulk-Fill Volume:** Volume of DMSO (in microliters) needed to dilute the source compound.

**New Concentration:** Concentration of the intermediate compound. The Echo Dose-Response software automatically calculates this concentration.

**Note:** The range of new concentrations created depends on the working range of the source plate that is used. The plate with the widest range is **Labcyte 384PP StdV**, which has a working range from 20  $\mu$ L to 50  $\mu$ L.

Working ranges on other source plates can be found at [www.Labcyte.com](http://www.Labcyte.com) (click **Products** menu, select **Consumables**, and open the Web page for Echo-qualified microplates).

**Step 3: Choose a name for the intermediate plate template:** Provides a name for the Intermediate Plate.

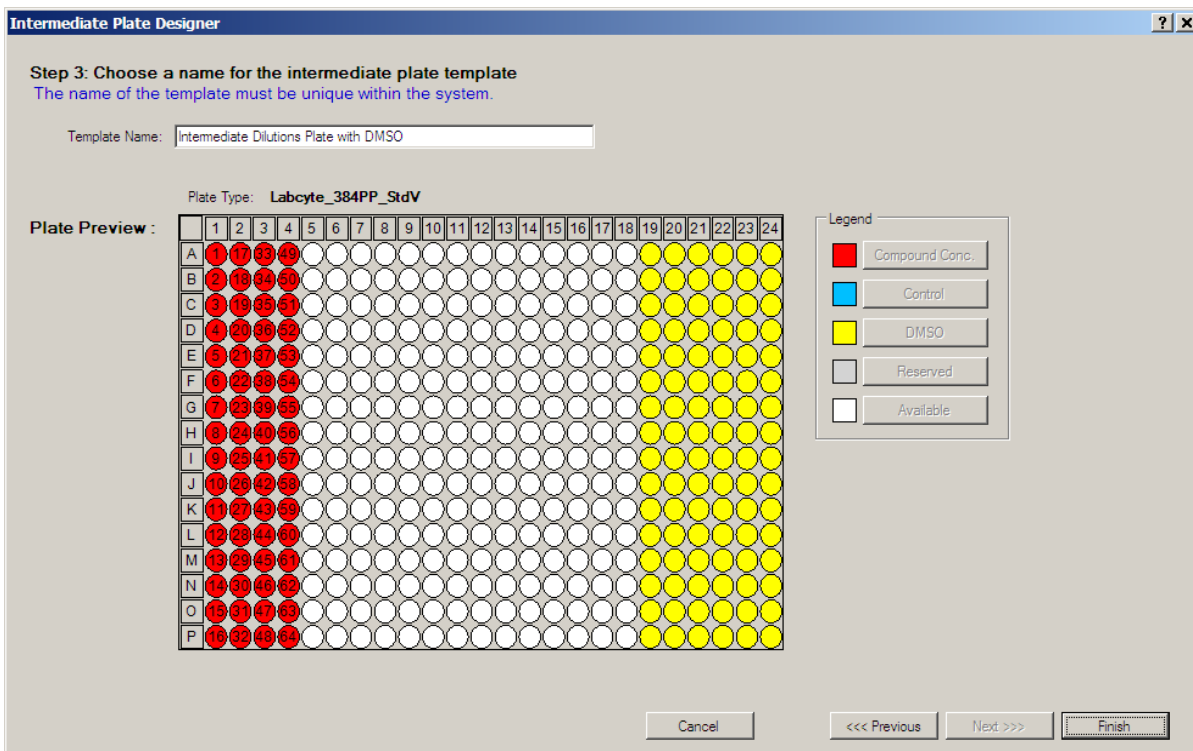


Figure 4-8: Source Plate Designer, Step 3

### 4.2.3 Destination Plate Designer

The **Add Destination** button begins the *Destination Plate Designer* wizard in a new window. This wizard helps you prepare a template of final compound concentrations that will be used to generate a dose-response curve.

**Step 1: Design the Destination Plate window.** Defines the destination plate type. Assigns wells to DMSO or blocks wells from compound transfer. See "*Step 1 Fields and Buttons*" below.

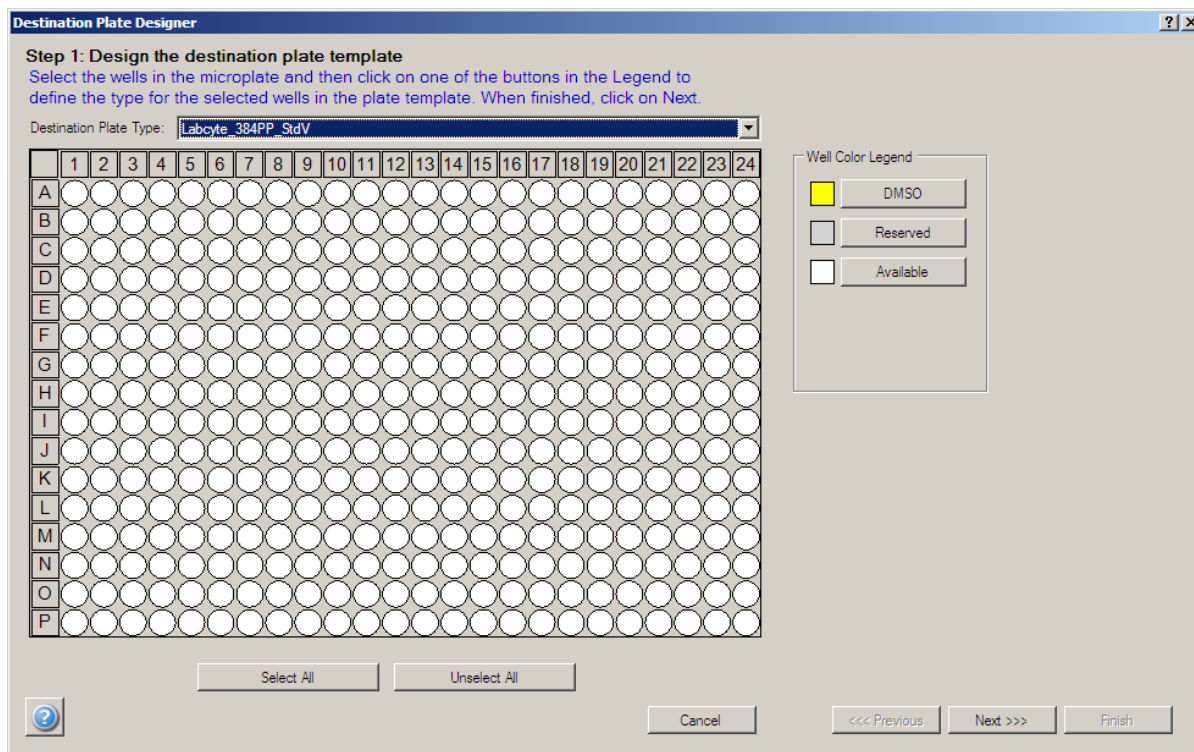
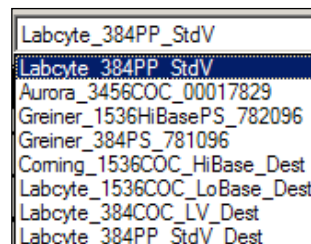


Figure 4-9: Destination Plate Designer

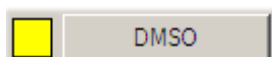
#### *Step 1 Fields and Buttons:*

**Source Plate Type:** Drop-down menu of available destination plates.

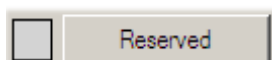
**Plate map of the selected destination plate:** Well positions that can be clicked and assigned to the components in the *Well Selection Action* box.



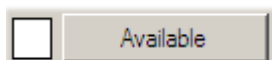
**Well Selection Action:** Components that can be assigned to the selected well positions.



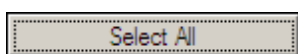
Assigns wells to hold DMSO for bulk-filling compound wells in the intermediate plate or assay wells in the destination plate.



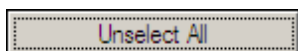
Prevents liquid transfer to or from the selected wells.



By default, all well positions are available. Use this button to deselect well positions that have been previously selected.

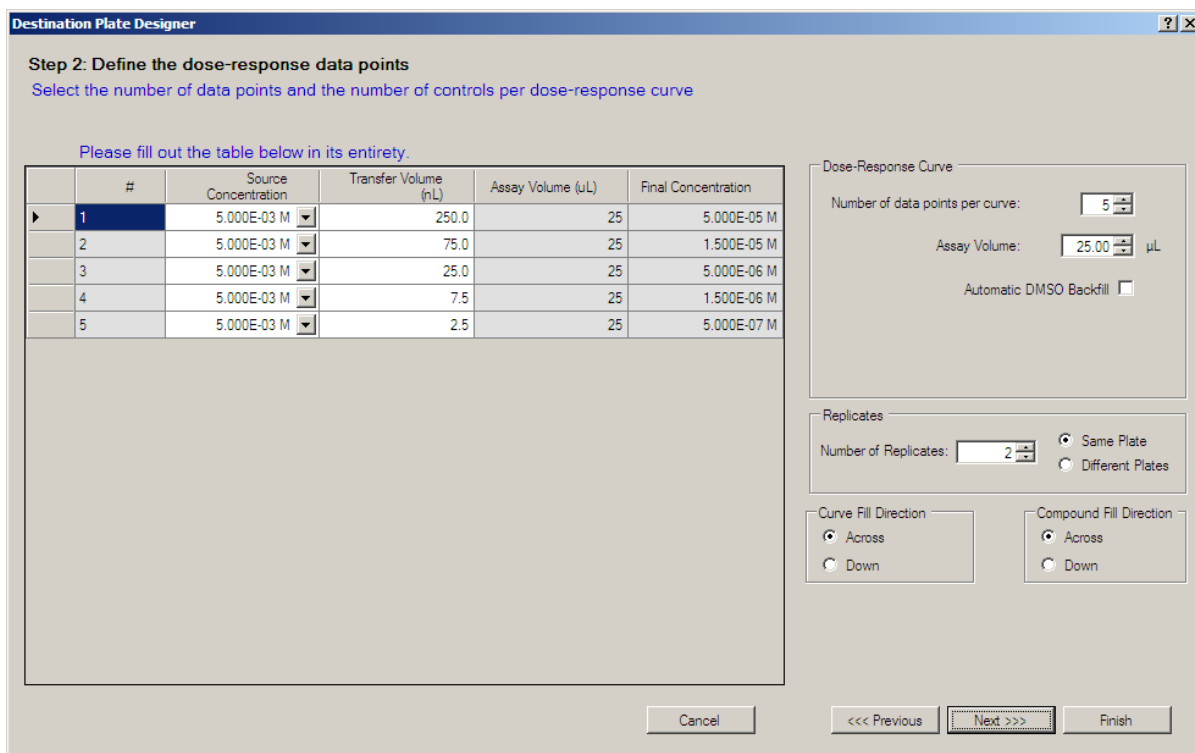


Selects all wells in the plate template for quick well assignment. Same action as clicking the corner cell above well **A**.



Unselects all wells in the plate template that were previously selected.

**Step 2: Define the Dose-Response Data Points window.** Selects the number of intermediate concentrations. This number corresponds to the concentrations fields in the table. See "Step 2 Fields".



**Figure 4-10: Destination Plate Designer, Step 2**

**Step 2 Fields:**

**Data Point Table:** Table of compound concentrations, transfer volume, assay volume, and calculation of final concentration

**Dose-response curve:** Data fields to specify the number of data points in the dose-response curve, assay volume needed for each well, and option to automatically backfill the wells to the assay volume.

**Replicates:** Data fields to specify the number of curve replicates on the same plate or across separate plates (single plate per curve and its replicates). In the same plate option, replicates can be added after the last curve in the plate or placed adjacent to the original curve.

**Curve Fill Direction:** Transfers concentrations of the same compound across or down the plate.

10 curve concentrations for compound 1



Figure 4-11: Curve fill across

**Compound Fill Direction:** Transfers different compounds across or down the plate.

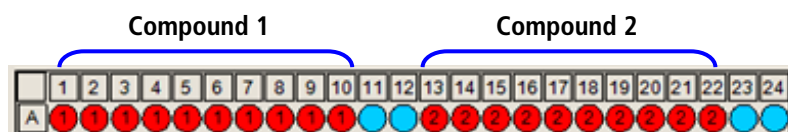


Figure 4-12: Compound fill across

**Step 3: Choose a name for the destination plate template window.** Provides a name for the Destination Plate and displays the final layout.

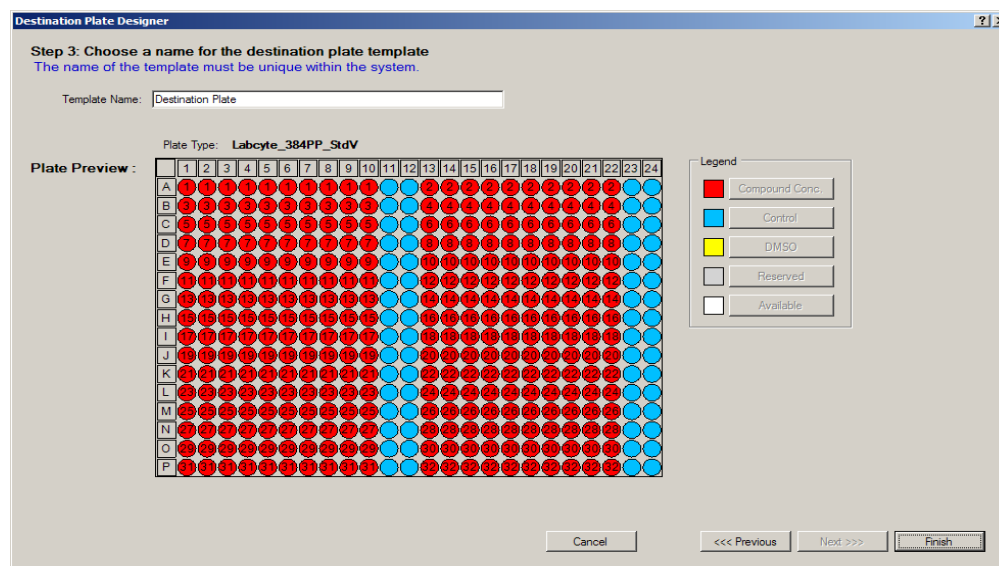


Figure 4-13: Destination Plate Designer, Step 3

## 4.3 Protocol Run

The *Run Options window* enables you to run a simulation of the transfer protocol or run the transfer protocol on the Echo liquid handler. If you are not connected to an instrument, this window also provides a connection dialog box.

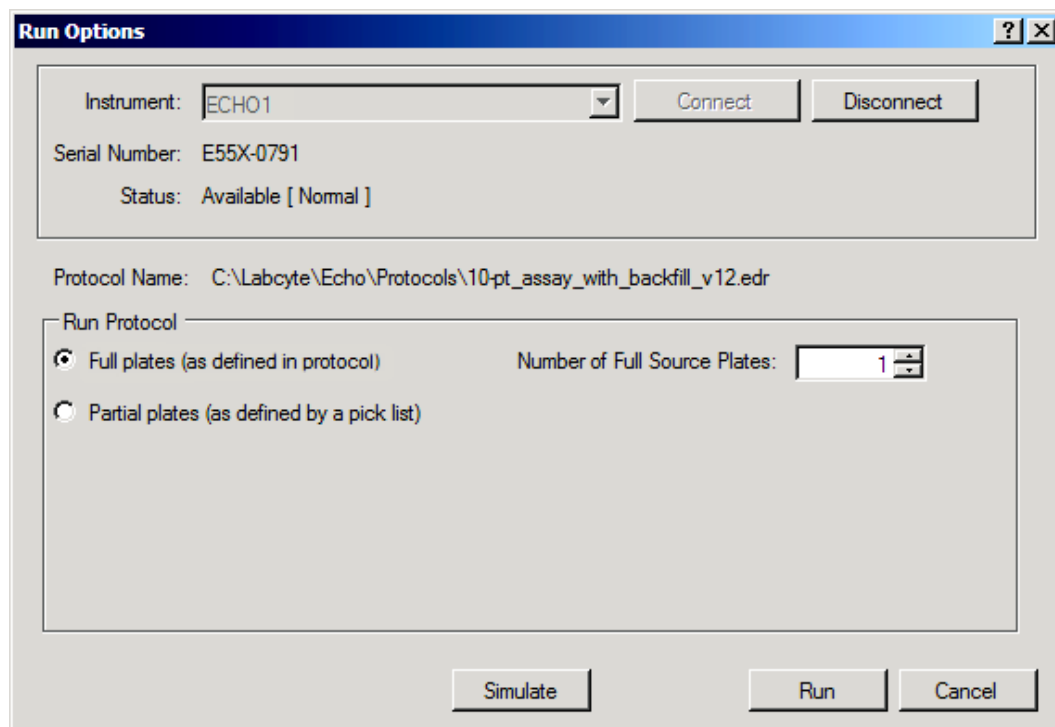


Figure 4-14: Run Options window

### **Control Buttons:**

**Connect:** Allows connection to the Echo liquid handler that is selected in the Instrument field. When the software is connected, it is "online".

**Disconnect:** Breaks the connection to the Echo liquid handler. When the software is disconnected, it is "offline".

**Simulate:** Opens a mock run window to simulate the protocol on the computer before performing the actual transfer.

**Full plates:** Plate templates defined in the transfer protocol. This is the default setting. The setting, **Number of Full Source plates**, allows you to process more than one source plate in the protocol run. See Example #2 in the Appendix.

**Partial plates:** Plate data imported from a pick list. The setting, **Remove Duplicate Entries**, instructs the software to remove duplicate wells that contain the same compound from the processing list. The setting, **Pre-process pick list in the order specified**, instructs the software to determine the source plate order in the pick list before the run.

### 4.3.1 Run Status

The *Run Status* window is a dynamic window that provides control buttons to run the protocol. It also contains various status windows to show the progress of the compound transfer, the state of the Echo liquid handler, and the final well positions and volume of the transferred fluid.

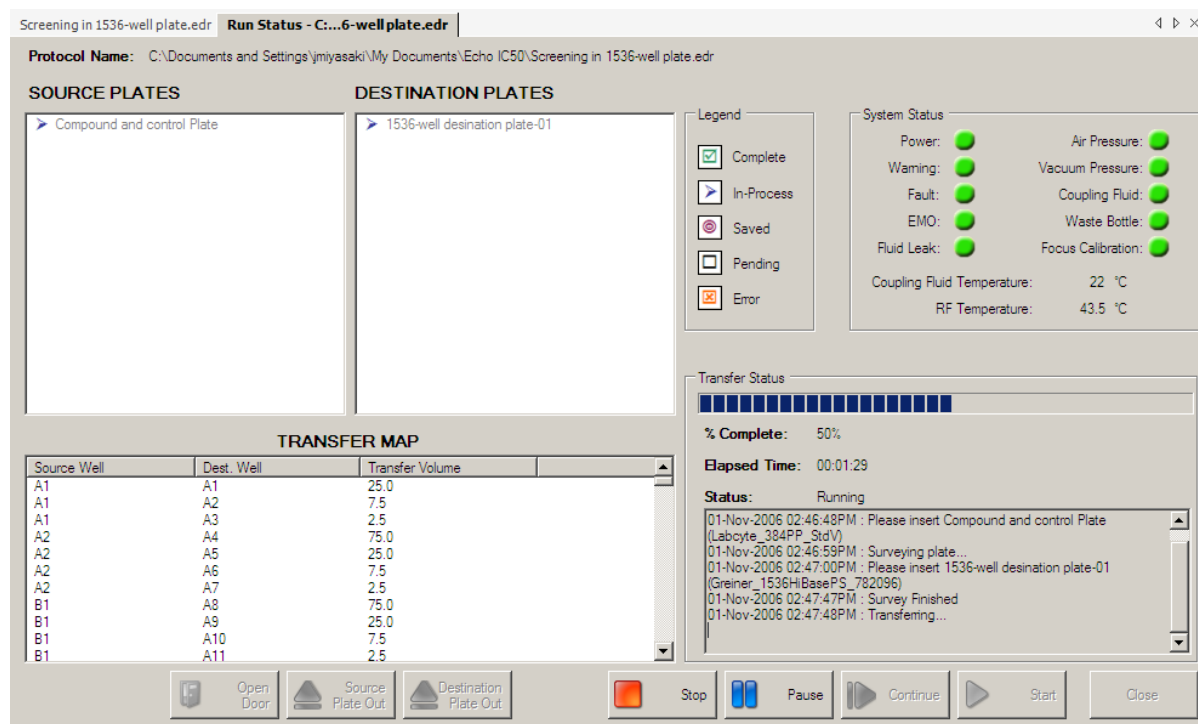
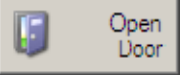
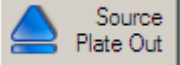
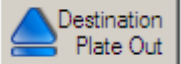

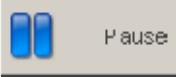
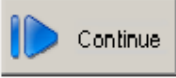
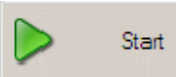


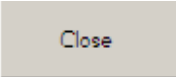
Figure 4-15: Run Status Window

**Control buttons:** Provides manual controls of the Echo liquid handler from the Echo Dose-Response software.

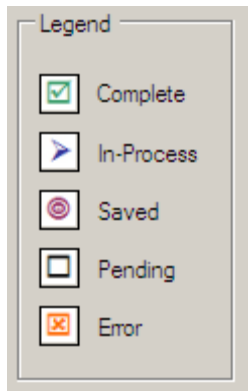
Control Buttons	Action
 Open Door	Opens the process door of the connected Echo liquid handler.
 Source Plate Out	Extends the source plate gripper.
 Destination Plate Out	Extend the destination plate gripper.
 Stop	Stops the transfer protocol that is in progress.

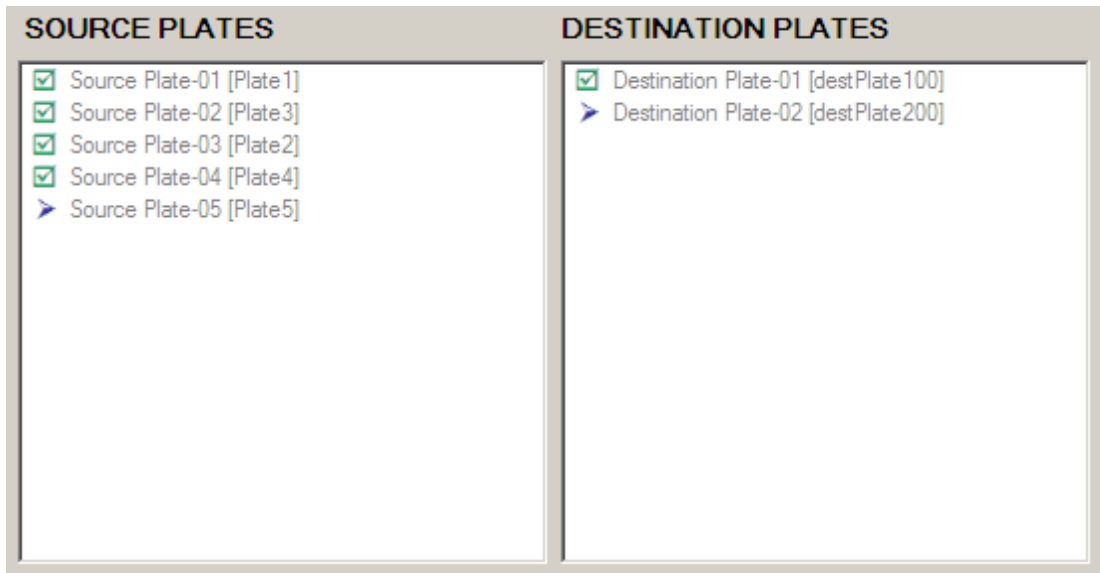


Control Buttons	Action
 Pause	Pauses the transfer protocol that is in progress.
 Continue	Continues the transfer protocol that is in progress.
 Start	Begins the transfer protocol.

 Close	Closes the <i>Run Status</i> window.
---	--------------------------------------

**Source and Destination Plate windows:** Shows the status of each plate as it progresses through the transfer protocol. The **Legend** interprets the symbols used in the status windows.

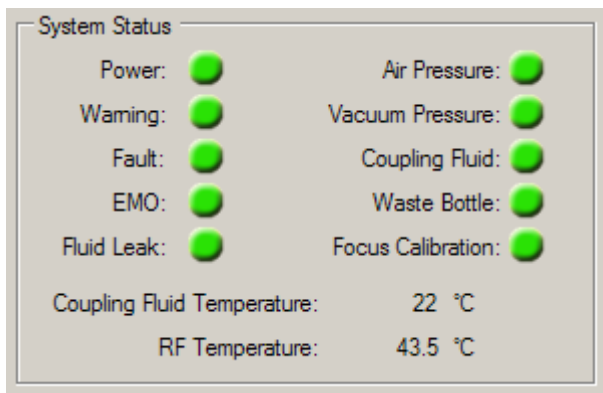




**Transfer Map window:** Shows the details of each well-to-well transfer.

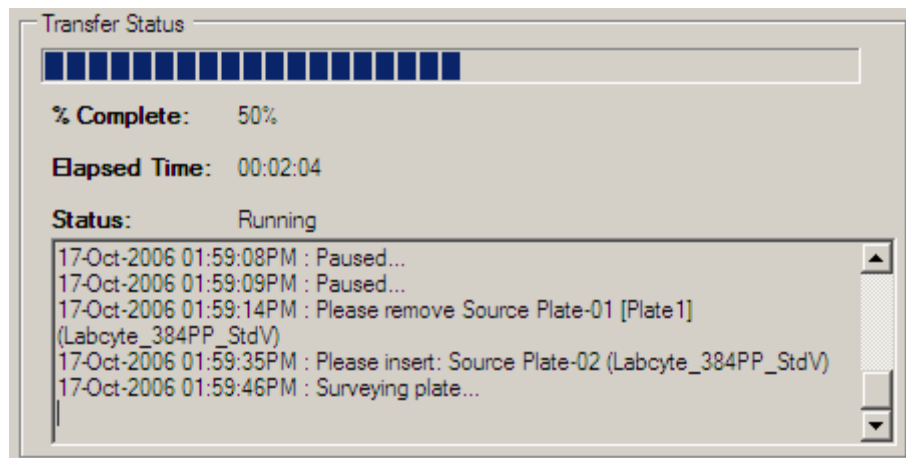
TRANSFER MAP		
Source Well	Dest. Well	Transfer Volume
I22	A6	2.5
E9	A7	2.5
H11	A8	2.5
L12	A9	2.5
G15	A10	2.5
O15	A10	2.5
G16	B1	2.5
F18	B2	2.5
C20	B3	2.5
F22	B4	2.5

**System Status window:** Shows the status of the Echo liquid handler during the transfer run. If a problem occurs, the alert will appear on this screen.



---

**Transfer Status:** Shows a progress bar of the transfer protocol, as well as percent completion and elapsed time. This section also provides a text window that displays all the actions that occur during the run.



### 4.3.2 Simulator

The simulator is an animation of fluid transfer for the selected protocol. The simulation is interactive and allows you to respond to a prompt box to insert and remove plates. Since this is a simulation, the prompt box appears for only 5 seconds, then the software automatically select the first available plate and continues to the next step.

The *protocol example* in Figure 4-16 is a simple source-to-destination plate transfer for a 5-pt dose-response curve. The components of the simulator window are described below.

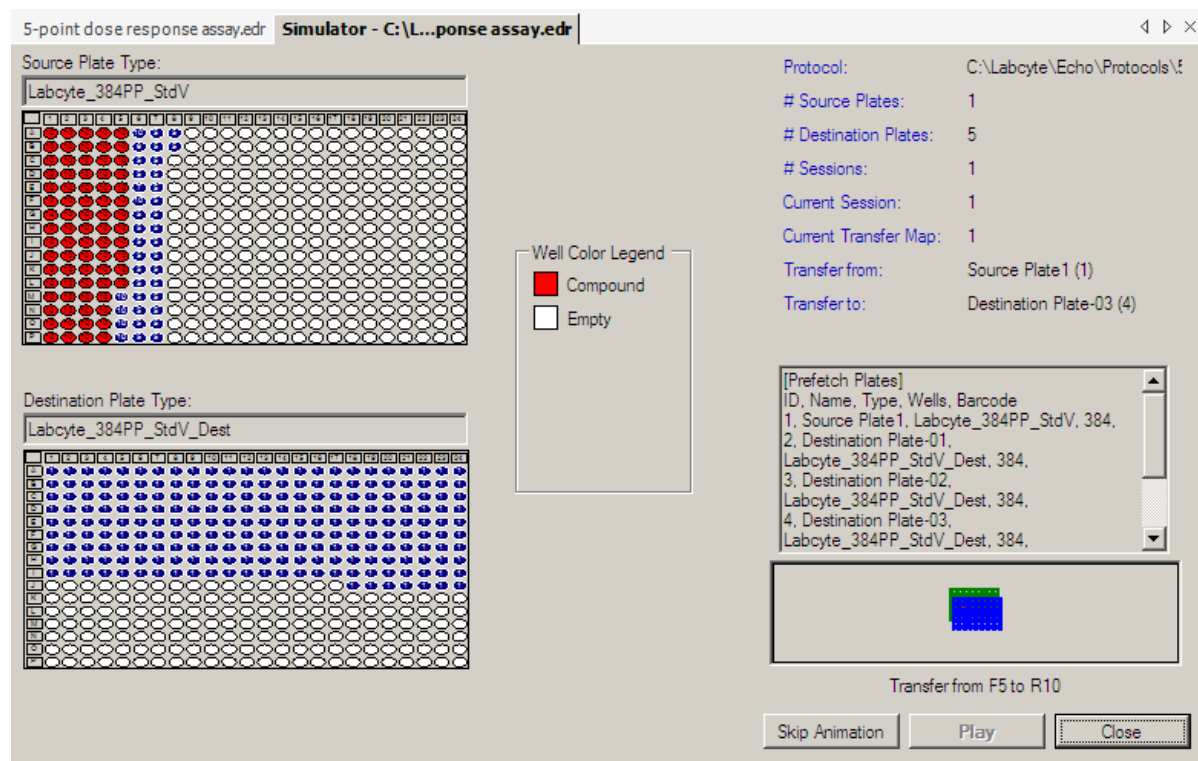
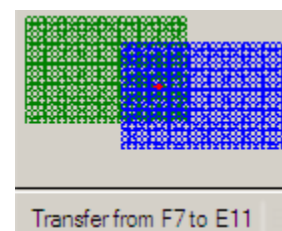


Figure 4-16: Simulator Window

The *transfer animation* (lower right corner of the window) simulates the movement of the destination plate (green) as it receives liquid from the source plate. Each source-to-destination well transfer is documented below the plates.



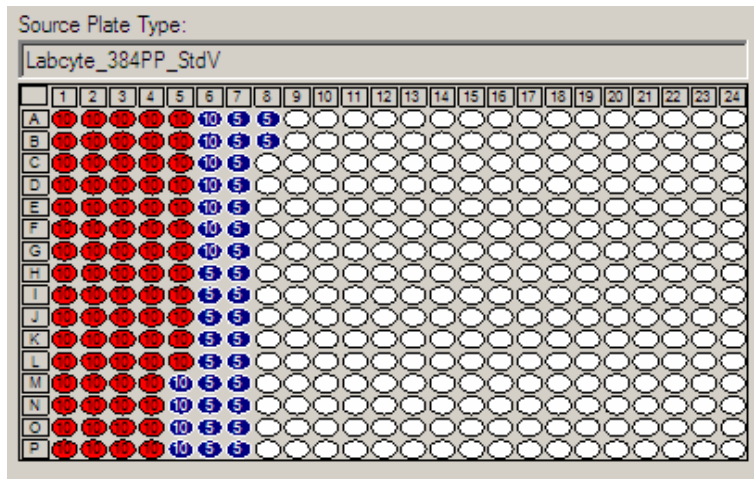
### **Control Buttons:**

**Skip Animation:** bypasses the animation of the current step and shows the completed step.

**Play:** begins the simulated compound transfer.

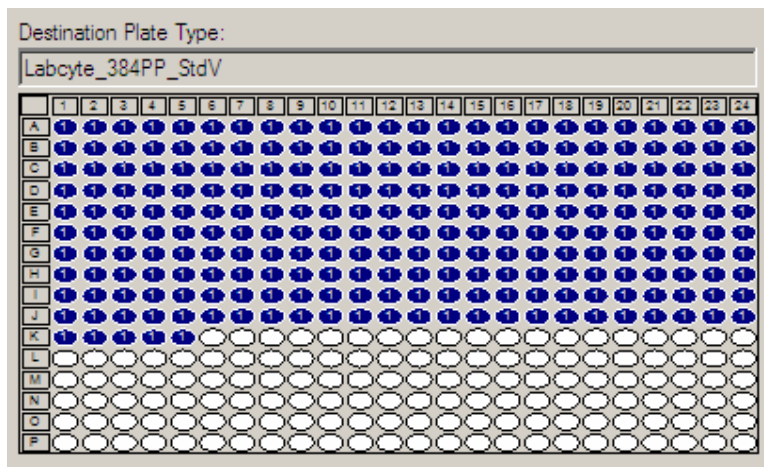
**Close:** closes the simulation window. If a simulation is in progress, you will need to cancel it before you can close the window.

The *Source Plate Type* graphic (upper left) shows the source plate that is selected for transfer.

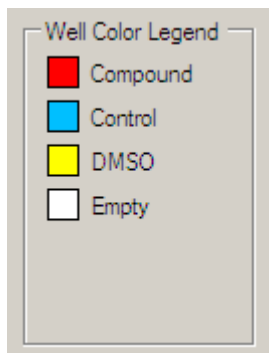


As the simulation runs, each source well shows the number of times the compound is aspirated. In this sample graphic the second source plate is in the process of being aspirated 5 times. The destination wells in red were transferred from the first source plate.

The *Destination Plate Type* graphic (lower left) shows each well that receives a dispense and the number of dispenses that are received. In this graphic the destination plate receives a single dispense. When the dispenses are complete, the well color will turn red.



The **Well Color Legend** (middle) shows the colors that correspond to the **compound** being transferred (red), **control** (cyan), **DMSO** (yellow), and **Empty** (wells that have not been aspirated or dispensed). Dark blue wells represent wells that are in the middle of the transfer process. When the transfer is complete, the well changes to the color that matches the Well Color Legend.

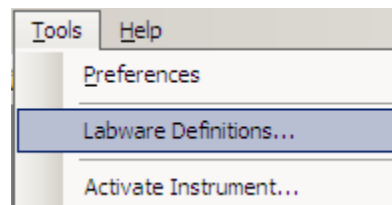


The **Protocol** information box (upper right) provides details about the transfer protocol, such as the number of plates that will be needed. As the simulation proceeds, this box is updated to show which plates are currently in use.

Protocol:	C:\Labocyte\Echo\Protocols\5-point dose
# Source Plates:	1
# Destination Plates:	5
# Sessions:	1
Current Session:	1
Current Transfer Map:	1
Transfer from:	Source Plate1 (1)
Transfer to:	Destination Plate-03 (4)

## 4.4 Labware

The Echo Dose-Response software relies on the labware definitions resident in the Echo liquid handler database. You can add new plate definitions or manage the existing ones by selecting **Labware Definitions** from the **Tools** menu.



### 4.4.1 Plate Type List

The *Plate Type List* opens to display the source and destination plates that are available from the Echo liquid handler database. If you are not connected to an Echo instrument, the list is cached locally (stored in the Echo Dose-Response software).

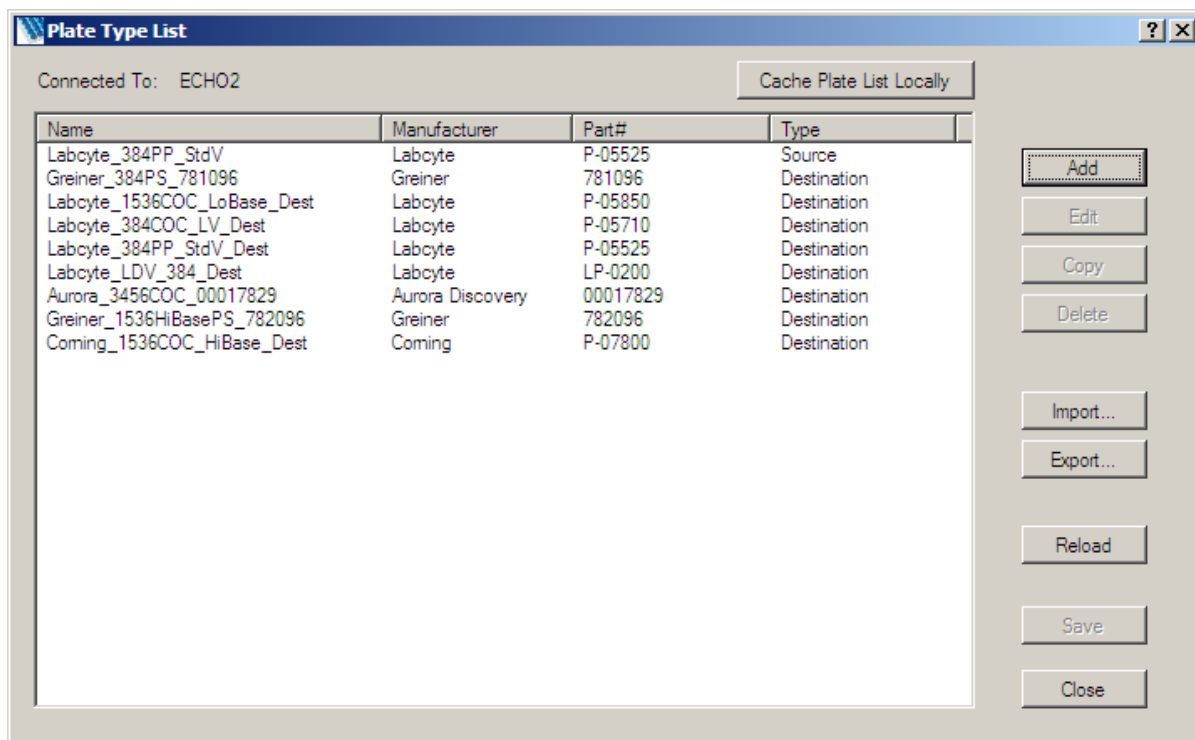


Figure 4-17: Plate Type List

**Note:** If you have installed the Echo Dose-Response software and receive an error message followed by a blank Plate Type List, you will need to connect to an Echo instrument and cache the plate list from the Echo instrument.

### ***Command Buttons:***

**Cache Plate List Locally.** Click this button to save the plate list from the Echo liquid handler to the Echo Dose-Response software.

**Add** a plate definition. Only a destination plate can be added. Refer to one of the following options:

- ◆ Add a labware definition from the Echo Dose-Response software
- ◆ Add a plate definition from the Echo liquid handler software and reload the updated labware definition to the Echo Dose-Response software.

**Edit** a plate definition. Select a plate definition and click the **Edit** button.

- ◆ Source plate definition: Only the barcode location of the source plate can be edited.
- ◆ Destination plate definition: Edit any setting in the definition.

**Copy** a plate definition. Select a plate definition and click the **Copy** button. You can copy a source or destination plate definition to create a destination plate definition.

**Delete** a plate definition. Select a plate definition and click the **Delete** button. You can delete a destination plate definition but not a source plate definition.

**Import** a single plate definition or group of definitions from a specific file location. The file must have an `.elw` extension to be imported.

**Export** a single plate definition or group of definitions.

**Reload** the plate types that have been edited or deleted since the plate type list was last saved.

**Save** any changes you make to the plate type list, such as adding a new plate definition.

**Close** the *Plate Type List* window.



## 4.4.2 Plate Type Editor

The *Plate Type Editor* window is similar to the *Plate Specification* window in the Echo liquid handler software.

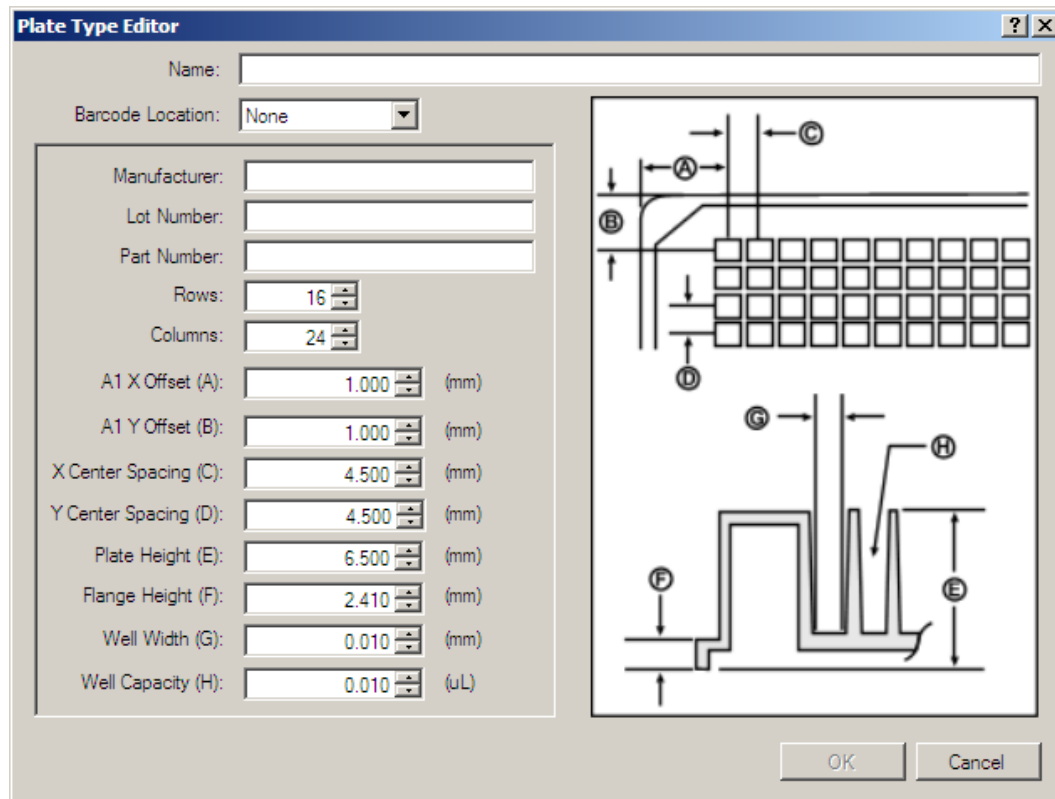


Figure 4-18: Plate Type Editor

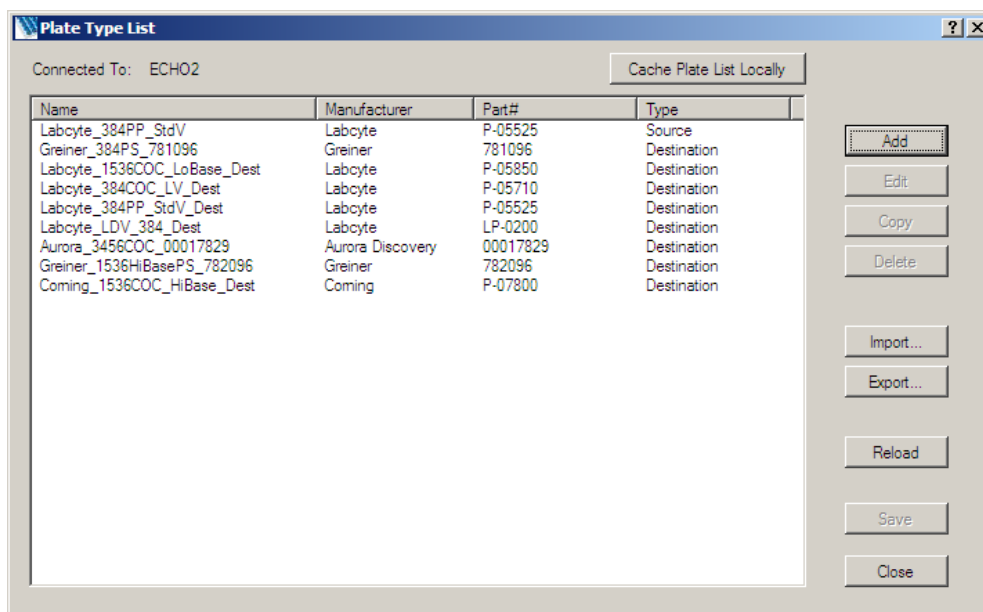
**Source plate definitions:** Source plates require very exacting specifications and calibration to accurately transfer nanoliter volumes; therefore, they are defined specifically for the Echo liquid handlers and tested at Labcyte (Echo-qualified). For this reason, source plates cannot be defined by the user. For existing source plates, only the barcode location can be edited. Contact Labcyte to add more source plates.

**Destination plate definitions:** The Echo liquid handler is programmed with several compatible destination plates; however, you can add destination plates through the Echo Dose-Response software or the Echo liquid handler software.

See "Labware" on page 73 for more information on plate definition. See online Help for procedures on adding, editing, or deleting labware.

**Fields:**

**Name** User-defined name to identify the plate. This name will be listed in the *Plate Type List* window



**Barcode Location** Location of barcode label on the destination plate:  
None  
Left  
Right  
Long

**Manufacturer** Name of the plate manufacturer

**Lot Number** Lot number assigned by the plate manufacturer

**Part Number** Part number assigned by the plate manufacturer

**Rows** Number of rows in the microplate

**Columns** Number of columns in the microplate

**AI XOffset (A)\*** Distance from left outside edge to center of first column where The left edge of the part will be defined as the two 12.7 mm areas (as measured from the corners) as specified in ANSI/SBS 1-2004 (internet link). Valid range: 0.0 to 128.0 mm.

\* The items marked A-G are measurements that need to be taken by hand or filled in from the manufacturer's specifications. These dimensions and further details on their definitions can be found at <http://www.sbsonline.com/msdc/approved.php>.

**A1 Y Offset (B)**

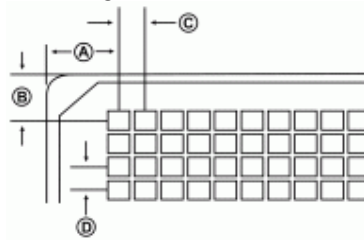
Distance from top outside edge to center of first row where The top edge of the part will be defined as the two 12.7 mm areas (as measured from the corners) as specified in ANSI/SBS 1-2004 (internet link). Valid range: 0.0 to 86.0 mm.

**X Center Spacing (C)**

Column spacing: Each following row/column shall be an additional X.Y mm in distance from the top/left outside edge of the plate as specified in ANSI/SBS 4-2004 (internet link). Valid range: 0.05 to 9.0 mm.

**Y Center Spacing (D)**

Row spacing: Each following row/column shall be an additional X.Y mm in distance from the top/left outside edge of the plate as specified in ANSI/SBS 4-2004 (internet link). Valid range: 0.05 to 9.0 mm.



**Plate Height (E)**

The overall height of the plate as specified in ANSI/SBS 2-2004 (internet link). Valid range: 6.5 to 4.5 mm.

**Flange Height (F)\*\***

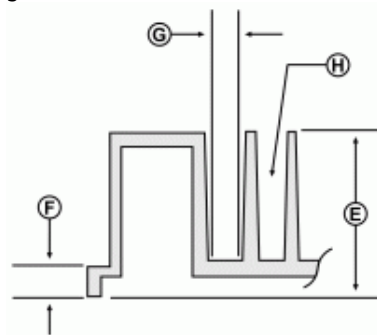
The height of the flange (skirt) as specified in ANSI/SBS 3-2004 (internet link). Valid choices: 2.41 mm, 6.10 mm, and 7.62 mm. See Note 2 below.

**Well Width (G)**

The width of the well opening at the bottom (not an SBS specified dimension). Valid range: 0.0 to 86.0 mm.

**Well Capacity (H)**

The overall capacity of the well in microliters. Valid range: greater than 0.0 uL.



\*\* Currently, the Echo liquid handler does not use dimensions F (flange height) and H (well capacity). Any value entered for flange height or well capacity is ignored.

### 4.4.3 Plate Export

The *Export Plates* window displays the plates currently available in the Echo Dose-Response software. You can export one or more plates to a file (.elw file extension) for use in another application.

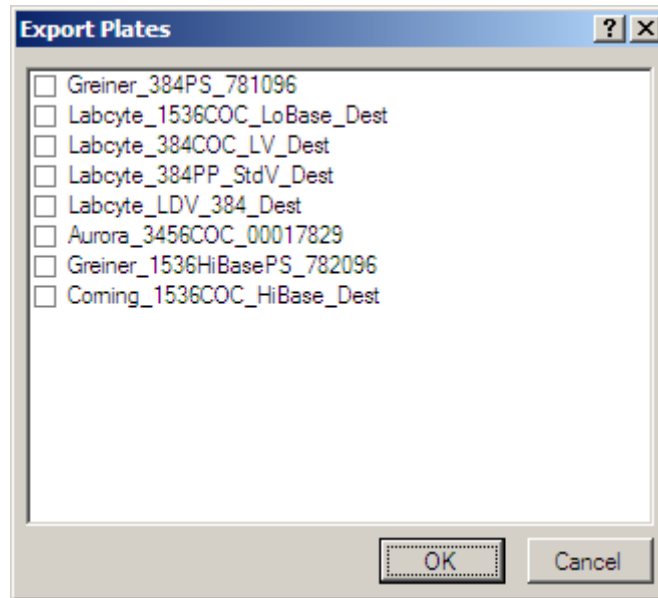
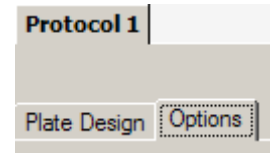


Figure 4-19: Export Plates Window

---

## 4.5 Protocol Options

The *Protocol/Options* window is accessible by clicking the *Options* tab behind the *Plate Design* tab. This window enables you to customize the survey, output, and reporting options for the Echo Dose-Response software. These settings are automatically used for any protocol you create.



The *Protocol/Options* window displays the following protocol and output options.



**Figure 4-20: Options Window**

---

**Note:** The options settings are similar to the Preferences/Options window, but apply only to the displayed protocol.

---

## 4.5.1 Survey History

The *Survey History* function stores survey data for source plates. When the source plate is returned to the Echo liquid handler, the Dose-Response software recalls and uses the stored survey data instead of resurveying the plate.

This feature can reduce processing time during a protocol run—particularly if the source plate is swapped several times during a protocol run. You can specify the length of time that the survey data is retained, from a few minutes to never resurvey.

The software stores the survey data by plate barcode; therefore, the data is independent of the protocol and can be used repeatedly in different protocols until the expiration time.

---

**Note:** Various factors can affect the content of the source wells, such as DMSO concentration, humidity, and exposure time during the transfer run; therefore, consider these factors when you select an expiration time. Read the Labcyte application note on how to calculate TTL.

---

The **Survey History** function is located in the *Options* window:

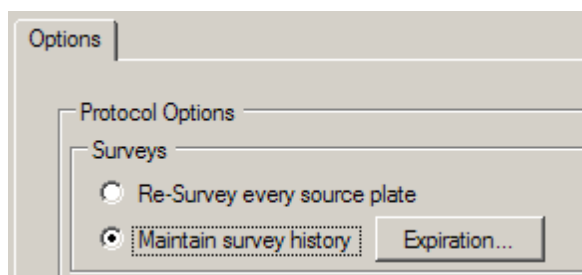
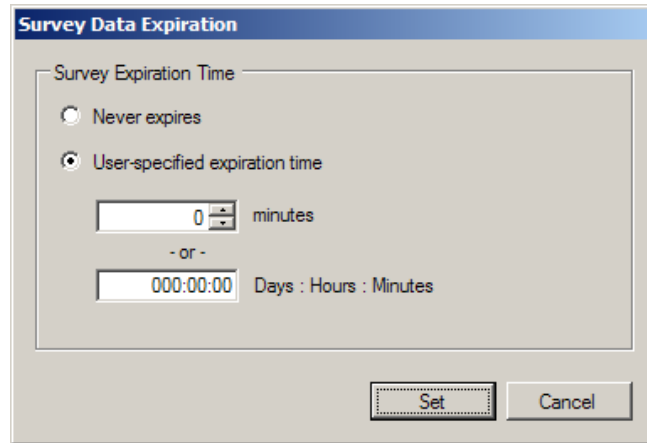


Figure 4-21: Survey option

---

**Control Buttons:**

- **Re-survey every source plate:** Default setting that automatically surveys the source plate every time the protocol is run.
- **Maintain Survey History: Expiration** button opens the *Survey Data Expiration* dialog box.



**Figure 4-22: Survey Data Expiration**

**Control Buttons:**

- **Never expires:** Existing survey data is always re-used.
- **User-specified expiration time:** Existing survey data is re-used for the time that is specified in *minutes* or *days:hours:minutes*.

---

**Notes:**

- If a stored survey does not cover the plate area that is required by the protocol (for example, some of the wells were empty when the plate was originally surveyed, but then filled later on), the software will survey the missing wells and add this data to the stored survey data.
  - The stored survey data is instrument-specific. For example, if a source plate is surveyed in instrument A, the stored survey data cannot be used in instrument B.
  - Although survey history can be used indefinitely, it is not recommended.
-

## 4.5.2 Output Files

The *Output Files* box in the *Options* window organizes the survey and transfer results according to the following criteria:

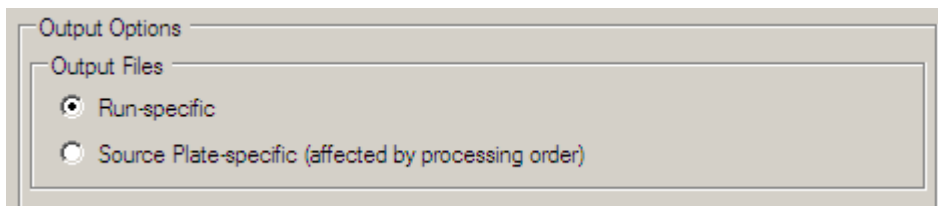
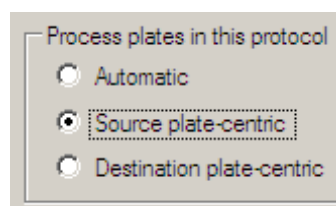


Figure 4-23: Output Files options

**Run-specific:** All protocol data is stored in one file, in the order that the results are generated.

**Plate-specific:** Protocol data is stored in separate files, based on source or destination plates (depending on which process order you specified in the *Plate Design* window).



## 4.5.3 File Type

Survey and transfer results can be saved to the following file formats that are compatible with most laboratory information systems:

- ◆ **XML** (extensible markup language)
- ◆ **CSV** (comma separated values)
- ◆ **TXT** (text file)

The results will be saved in the formats you have checked, and stored in the directory you specify in the *Directory* option.



---

## Report Format

The survey and transfer reports can be customized by clicking the **Format** button in the *Protocol Options* window.

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**Note:** The DEFAULT settings in the Transfer and Survey Reports are from the *Preferences/Options* window.

---

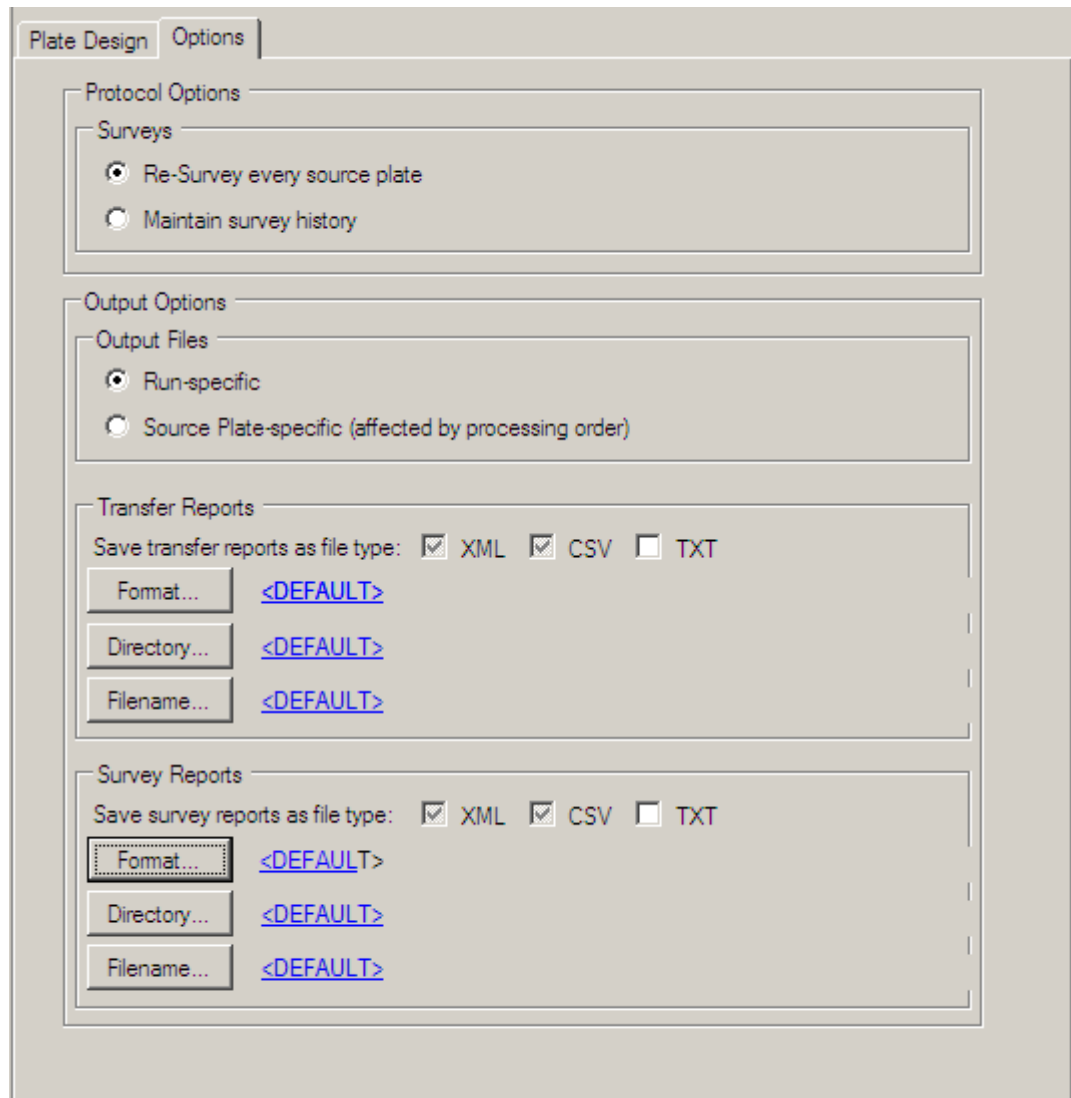


Figure 4-24: Report Format Options

The *Output Format Builder* window shows all the report variables (left selection box) that can be added to the report format (right selection boxes). The list of variables changes with the report area that you select.

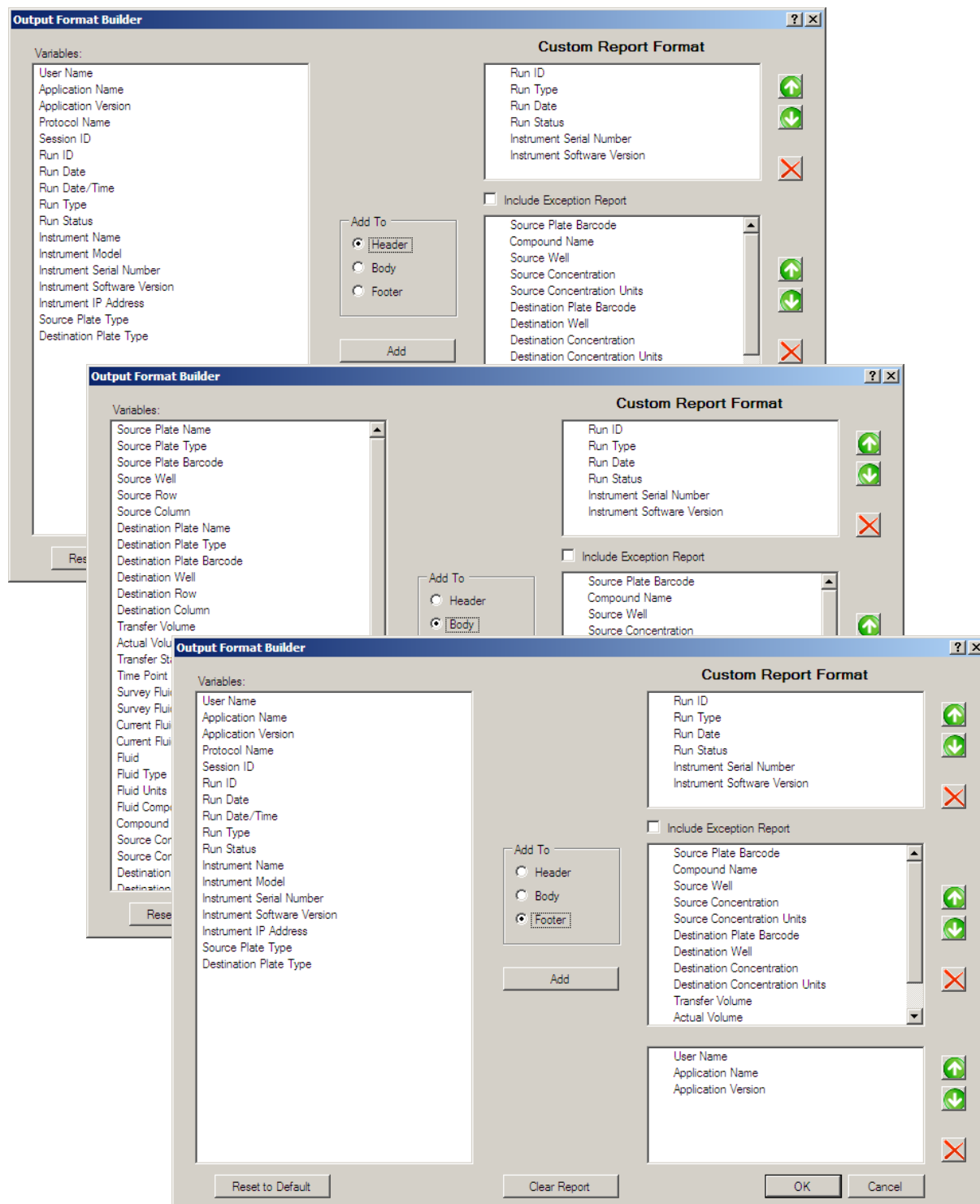


Figure 4-25: Transfer Output Format Builder

---

## Report Directory

The default *report directory* for both surveys and transfers is **C:\Labcyte\Echo\Reports**. You can specify a different directory by clicking the **Directory** button and entering a new location. Use the **Select Folder** button to browse to a different directory.

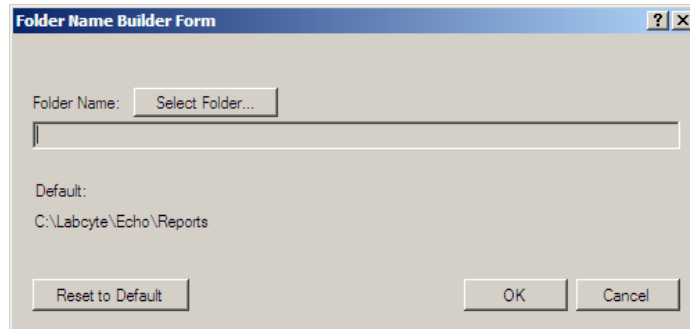


Figure 4-26: Folder Name Window

## Report Filename

The Echo Dose-Response software uses the following default formats to report the survey and transfer results:

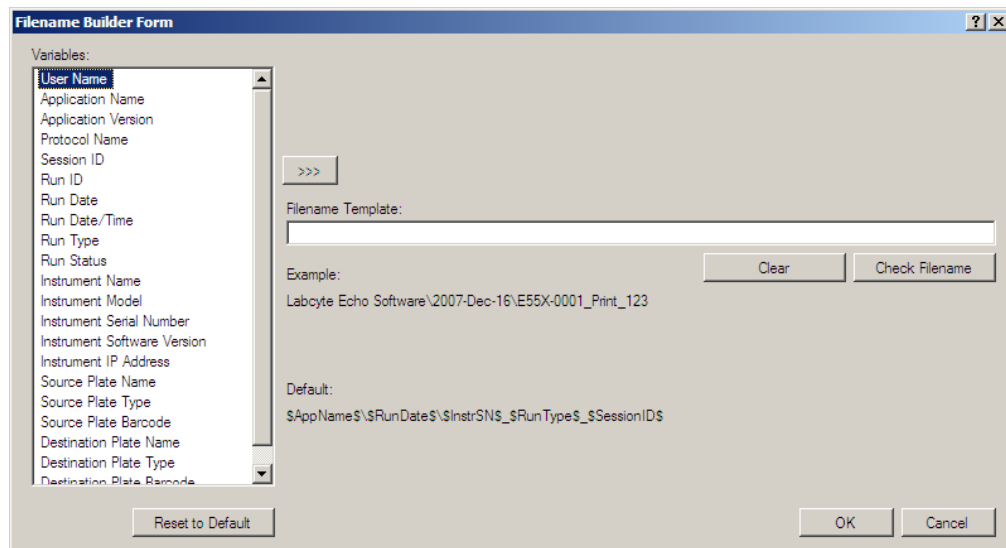
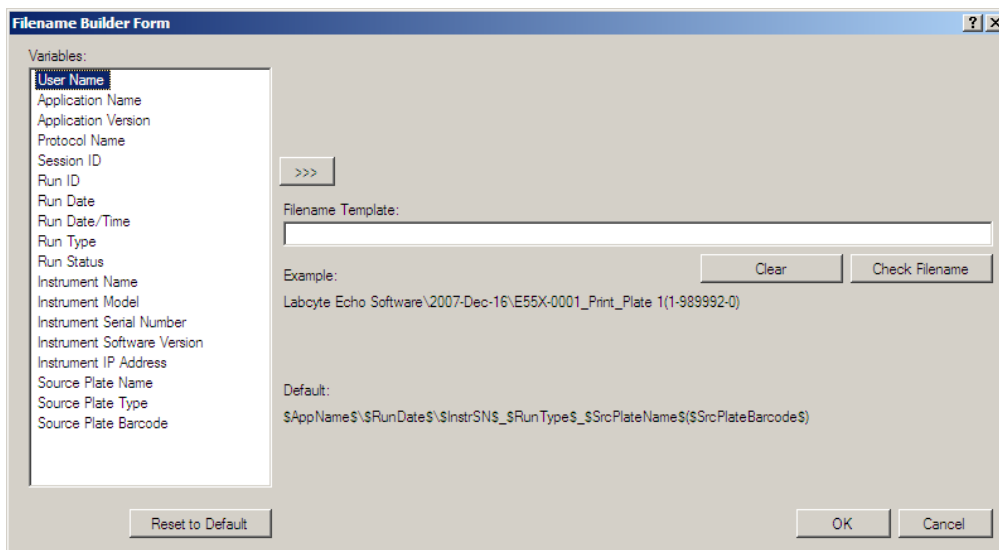


Figure 4-27: Transfer Report Filename

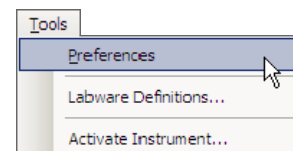


**Figure 4-28: Survey Report Filename**

You can specify a different transfer or survey filename by clicking the **Filename** button in the *Protocol Options* window. In *Filename Builder Form* window, select a variable and click >>> button to build the file name.

## 4.6 Preferences

The *Preferences/Options* window is accessible from the *Tools* menu. This window is very similar to the **Protocol Options** window, but with the following differences:



- Accessible from the Toolbar. When you click **Preferences**, the *Options* window\* opens to display the following protocol and output options:
- Settings automatically apply to all procedures unless you make changes in the *Protocol Options* window (report formats shown as "default").

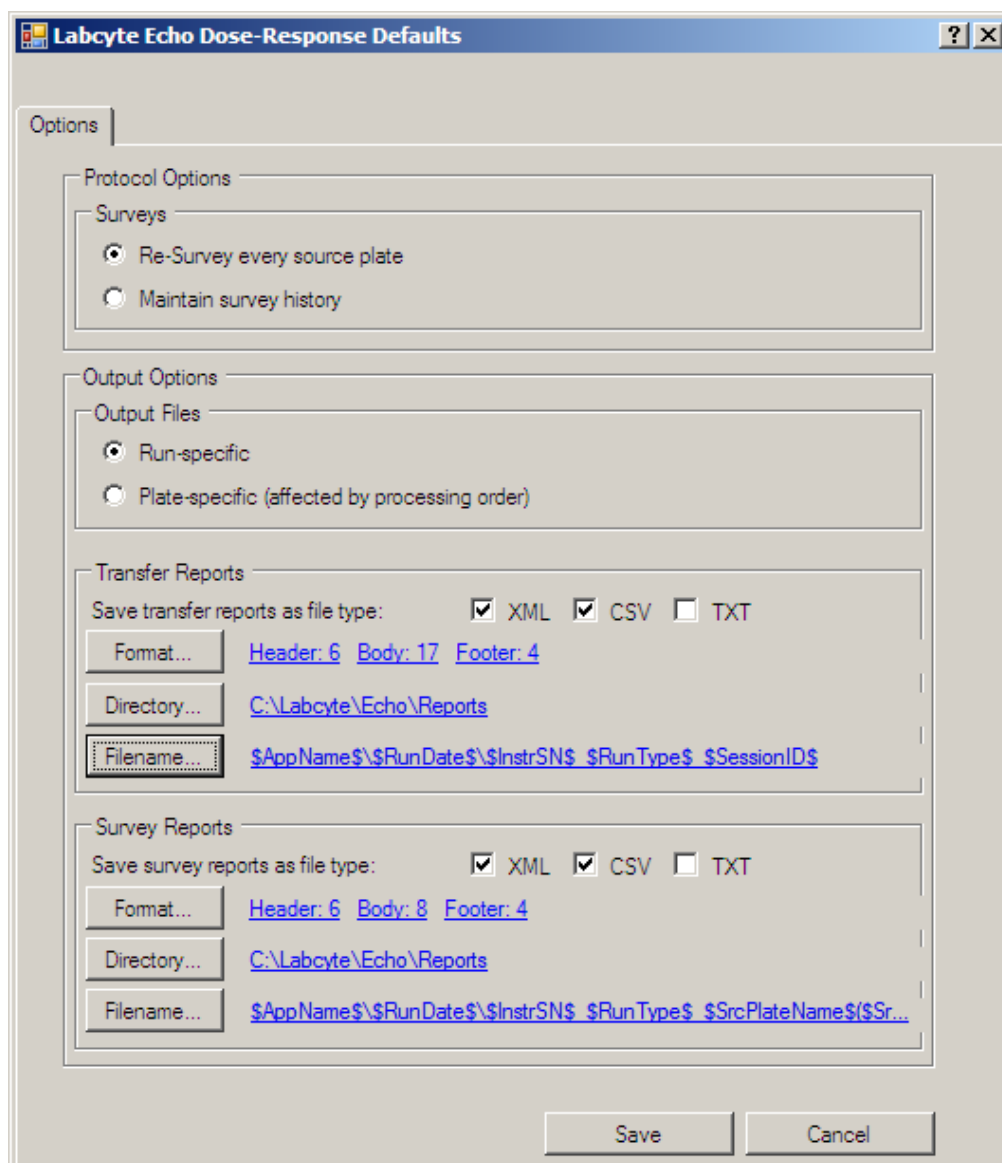


Figure 4-29: Preferences/Options Window

# Appendix


## Example 1: 12-pt curve with controls

### Plan Curve Data

This example is a 12-pt curve with controls.

Starting concentrations of your compounds:	5 mM
Layout for the source plate:	One source plate template containing compounds, controls, and DMSO
Layout for the intermediate plate:::	One intermediate plate with two dilutions from stock: 1/100 = 250 nL/25 µL 1/10,000 = 250 nL/25 µL
Final assay volume:	20 µL
Number of points needed for IC50 graph:	12-point curve

### Set up the transfer protocol

1. Start a new protocol. Click the new protocol icon  or select **New** from the *File* menu to open a new *Protocol* window.
2. Type the name and description (optional) of the protocol in the *Plate Design* window.

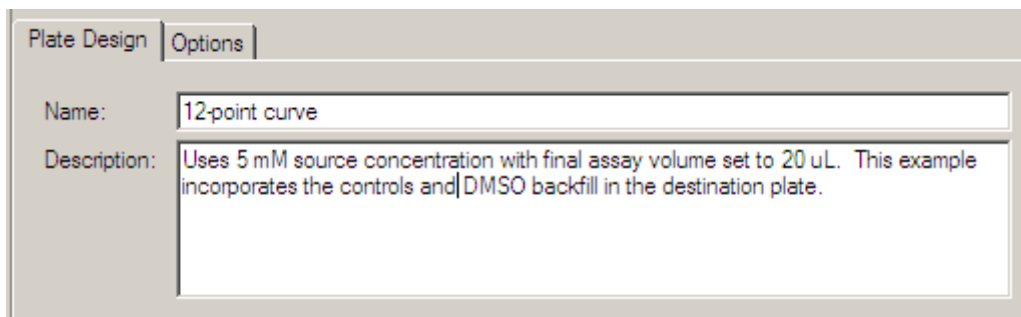
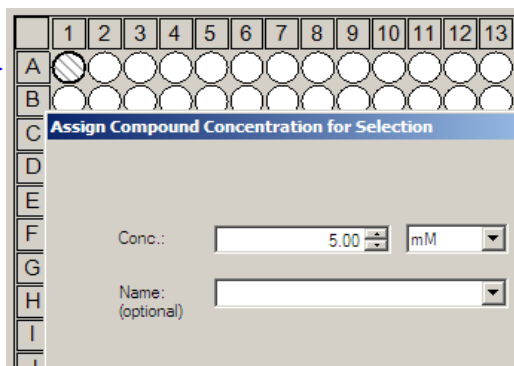


Figure A- 1: Protocol name and description

3. Design the source plate template using the following steps:
  - a. Click the **Add Source** button in the *Plate Design* window to open the source plate designer window.
  - b. Select the plate type of the source plate, for example, **Labcyte 384PP StdV**.
  - c. Select the wells that must be reserved for the controls and DMSO.

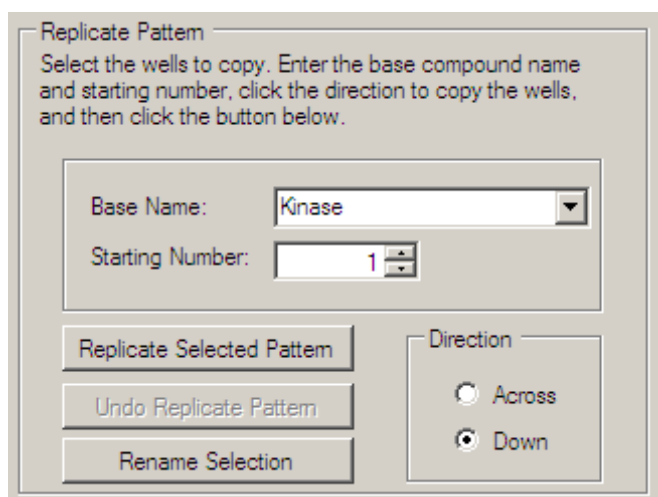
This example uses a source plate that contains compound, controls and DMSO for backfilling; therefore, some of the wells must be reserved before assigning compounds.

- d. Select the well that contains the first compound: **A1**
- e. Click the **Compound Conc.** Button to open the *Assign Compound Concentration for Selection* window.



**Figure A- 2: Assign Compound Concentration**

- f. Enter the compound concentration by typing the number or using the arrow keys. Modify the concentration units, if necessary.
- g. Leave the compound name blank and click **OK**.
- h. Enter the compound base name and starting number in the *Replicate Pattern* box.

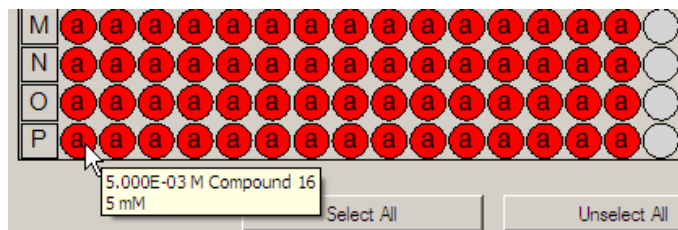


**Figure A- 3: Replicate selected pattern**

- i. Select the replicate direction for the compounds: **Down**

j. Click **Replicate Selected Pattern**.

The compound concentration (5 mM) will be replicated down the plate into all available wells. Compound 16 is at P1; Compound 240 is at P15 and is the last compound well because the remaining wells have been reserved.

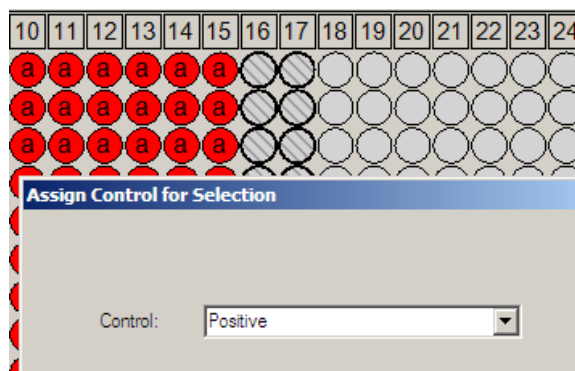


**Figure A- 4: Replication completed**

k. Select the reserved wells that will contain the first control:  
**columns 16, 17**

l. Click the **Control** button.

m. Type the control name in the prompt box: **Positive**. Click **OK**.

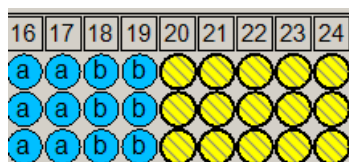


**Figure A- 5: Assign control wells**


n. Repeat for the negative control in **columns 18 and 19**.

The controls are labeled "a" and "b" on the plate map.

o. Select the remaining wells in the plate and click the **DMSO** button.



**Figure A- 6: Control and DMSO wells assigned**

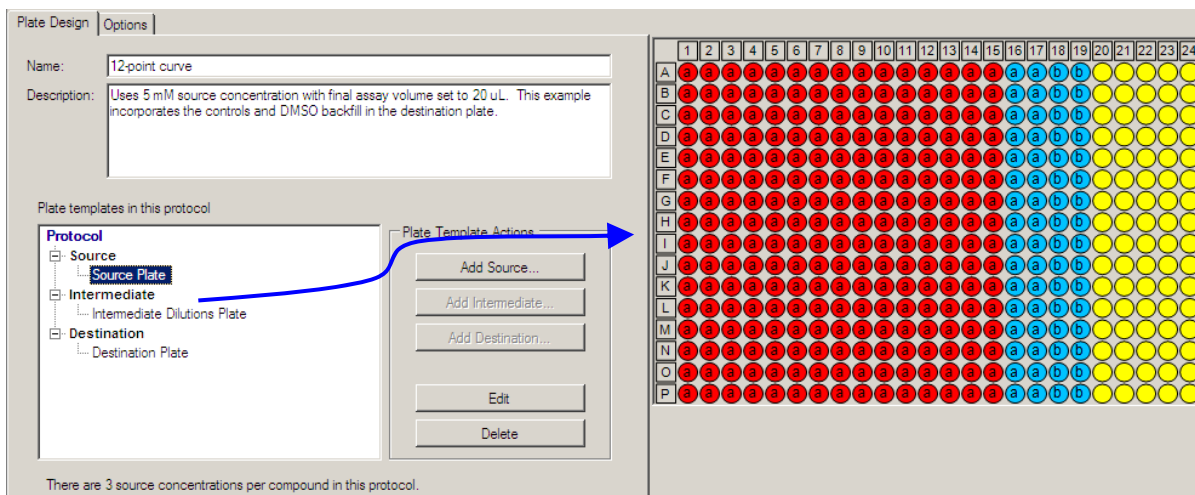
p. Enter a new source template name in the **Template Name** field or click the left arrow  button to accept the suggested name.



q. Click **Finish**.

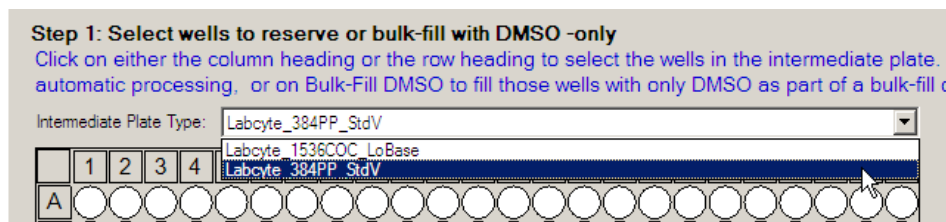
You are done with the source plate template.

To preview or edit this template from the *Plate Design* window, click **Source Plate** in the protocol tree. Click **Edit** to enter the template designer.



**Figure A- 7: Preview of source plate template**

4. Create the intermediate plate template.
  - a. Click the **Add Intermediate** button in the *Plate Design* window to open the *Intermediate Plate Designer* window.
  - b. Select the plate type of the intermediate plate.



**Figure A- 8: Select intermediate plate type**

---

**Note:** The intermediate plate must be an Echo-qualified source plate, since liquid will be transferred out of this plate.

---

- c. Select the bulk-fill direction: column or row.
- d. Select the wells that will be bulk-filled with DMSO.

---

**Note:** You can only select in the direction that is specified in the Bulk-Fill Direction box.

---

- e. Click **Next** to continue to Step 2.
- f. Enter the number of intermediate concentrations you want to create:  
In this example, enter **2**.  
Two concentration fields will be displayed.
- g. Select the compound concentrations from the drop-down menu.
- h. Enter the transfer volume for the first concentration.  
In this example, enter **2.5 nL**.
- i. Enter the volume of DMSO you want to bulk fill: **25  $\mu$ L**. The software will calculate the final concentration of the compound in the intermediate plate.
- j. Repeat steps e to g for the second intermediate concentration: **250 nL transfer volume, 25  $\mu$ L bulk fill**.

Intermediate transfers

How many intermediate concentrations would you like?

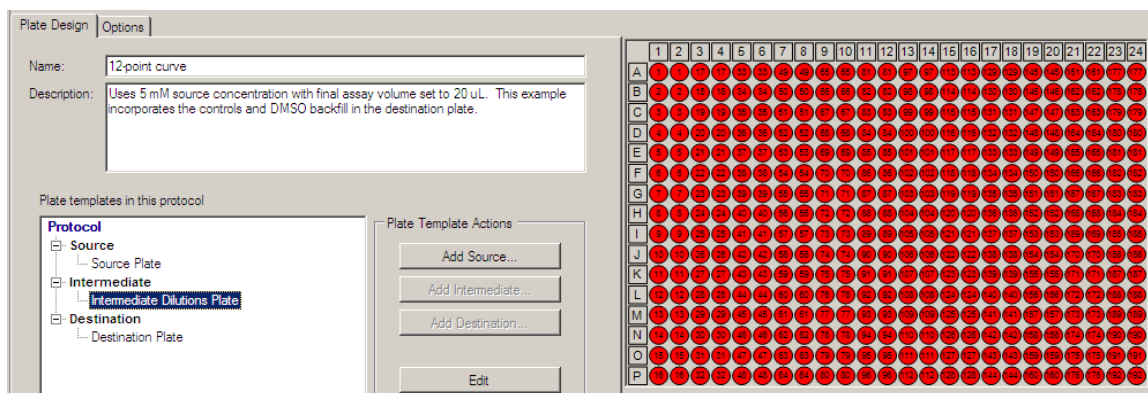
Source Concentration	Transfer Volume	Bulk-Fill Volume	New Concentration
5.000E-03 M	2.5 nL	25.00 $\mu$ L	5.000E-07 M
5.000E-03 M	250.0 nL	25.00 $\mu$ L	4.950E-05 M

**Figure 4-30: Determine compound concentrations.**

The intermediate plate template reserves two wells for each compound concentration.

- k. Click **Next** to enter an intermediate template name.
- l. Click the **Finish** button to return to the *Plate Design* window.

The intermediate template is done. To preview or edit this template from the *Plate Design* window, click **Intermediate Plate** in the protocol tree. Click **Edit** to enter the template designer.



**Figure A- 9: Preview of intermediate plate template**

**5.** Create the destination plate template.

- a. Click the **Add Destination** button in the *Plate Design* window to open the *Destination Plate Designer* window.
- b. Select the plate type of the destination plate. The controls will not be entered at this step because they will be included in the dose-response curve.
- c. Click **Next** to continue to Step 2.

This screen calculates the dose-response curve. The data you enter will define the layout of the final assay plate.

- d. Enter the following information in the *Dose-Response Curve* box (right dialog box):
  - Specify the Number of data points per curve: **14**
  - Enter the Assay Volume for all destination wells: **20 uL**
- e. Enter the following data in the *Data Point Curve* table (left dialog box):
  - Source concentration and control name from the drop-down menus.
  - Transfer volume for each data point.

The software calculates the backfill volume of DMSO and final compound concentration for each data point.

#	Source Concentration	Transfer Volume (nL)	Backfill Volume (nL)	Assay Volume (uL)	Final Concentration
1	5.000E-03 M	75.0	0	20	1.875E-05 M
2	5.000E-03 M	25.0	50	20	6.234E-06 M
3	5.000E-03 M	7.5	67.5	20	1.869E-06 M
4	5.000E-03 M	2.5	72.5	20	6.227E-07 M
5	4.950E-05 M	75.0	0	20	1.856E-07 M
6	4.950E-05 M	25.0	50	20	6.172E-08 M
7	4.950E-05 M	7.5	67.5	20	1.850E-08 M
8	4.950E-05 M	2.5	72.5	20	6.165E-09 M
9	5.000E-07 M	75.0	0	20	1.875E-09 M
10	5.000E-07 M	25.0	50	20	6.234E-10 M
11	5.000E-07 M	7.5	67.5	20	1.869E-10 M
12	5.000E-07 M	2.5	72.5	20	6.227E-11 M
13	positive	75.0	0	20	0.000E+00 M
14	negative	75.0	0	20	0.000E+00 M

**Figure A- 10: Dose-response data table**

**Note:** This example uses a 12-pt curve plus 2 controls for a total of 14 data points; therefore, the option to include controls is selected.

- f. Return to the Dose Response Curve box and enter the DMSO backfill volumes.
  - Select the **Automatic DMSO Backfill** option
  - Select the timing for the backfill: **Last** (after compound transfer). The software will calculate the volume of backfill needed and add it to the Data Point Curve table.
  - Select **Maximum Backfill Volume**. The default value is the highest transfer volume, which is **75 nL** in this protocol.
  - Select **Backfill offline for volumes greater than/equal to** and change the offline backfill volume to: **50 nL**.

Automatic DMSO Backfill

Perform Backfill:  First  Last

Maximum Backfill Volume: 75.0 nL

Backfill offline for volumes greater than/equal to:  
(Yellow denotes offline backfill) 50.0 nL

**Figure A- 11: Dose-response curve box**

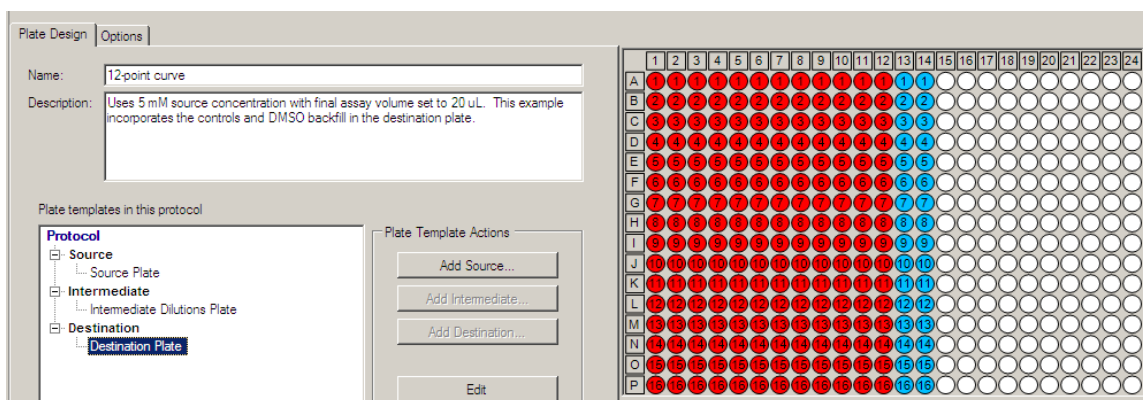
The data table automatically highlights in yellow the backfill volumes ( $\geq 50 \mu\text{L}$ ) that will be filled offline.

#	Source Concentration	Transfer Volume (nL)	Backfill Volume (nL)	Assay Volume ( $\mu\text{L}$ )
1	5.000E-03...	75.0	0	20
2	5.000E-03...	25.0	50	20
3	5.000E-03...	7.5	67.5	20
4	5.000E-03...	2.5	72.5	20
5	4.950E-05...	75.0	0	20
6	4.950E-05...	25.0	50	20
7	4.950E-05...	7.5	67.5	20
8	4.950E-05...	2.5	72.5	20
9	5.000E-07...	75.0	0	20
10	5.000E-07...	25.0	50	20
11	5.000E-07...	7.5	67.5	20
12	5.000E-07...	2.5	72.5	20
13	positive	75.0	0	20
▶ 14	negative	75.0	0	20

**Figure A- 12: Dose-response data table showing offline bulk-fill**

- g. Enter the following information in the *Replicates* box (right side):
  - Enter Number of Replicates: **3**
  - Select the **Same Plate** and **In Adjacent Wells** options.
  - Select the *Curve Fill Direction*: **Across**.
  - Select the Compound Fill Direction: **Down**.
- h. Click the **Next** button to enter a destination template name.
- i. Click **Finish**.

You are done with the destination plate template. To preview or edit this template from the *Plate Design* window, click **Destination Plate** in the protocol tree. Click **Edit** to enter the template designer.



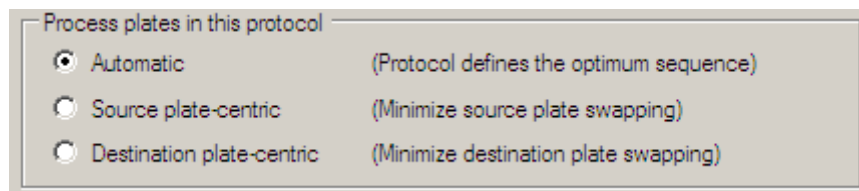
**Figure 4-31: Preview of destination plate template**

- In the *Plate Design* window, specify the order of processing the plates.

**Automatic:** The software selects the most efficient order of plate handling. This is the default setting and may be fine for most protocols.

**Source plate-centric:** The software minimizes the frequency of swapping the source plate. This option is useful if you need to complete the processing of the source plate and return it to storage.

**Destination plate-centric:** The software minimizes the frequency of changing the destination plate. This option is best if you need to complete the processing of the destination plate and put it in environmentally-controlled plate storage.



**Figure A- 13: Process plate options**

**Hint:** You can save processing time by turning on the **Survey History** function. This function saves survey data for the source plate and reuses it the next time that the source plate is returned to the Echo liquid handler. For more information, go to Survey History on page. 80.

- Check the protocol for missing or inconsistent data.  
Select **Error Check** from the *Protocol* menu.

The transfer protocol is done. Test the protocol in the simulation program before using it.

## Example 2: 12-pt curve using multiple source plates

### Plan Curve Data

This example is the same 12-pt curve protocol that was used in Example 1; however, it uses multiple source plates.

Starting concentrations of your compounds:	5 mM
Layout for the source plate: (4 source plates specified in Run Options)	One source plate template containing compounds, controls, and DMSO.
Layout for the intermediate plate:	One intermediate plate with two dilutions from stock: 1/100 = 250 nL/25 $\mu$ L 1/10,000 = 250 nL/25 $\mu$ L
Layout for the destination plate:	12-pt curve + 2 controls (2 replicates)
Final assay volume:	25 $\mu$ L
Number of points needed for IC50 graph:	12-point curve

### Set up the transfer protocol

Use the same procedure described in Example 1 to set up the protocol for Example 2. For this example, run 2 replicates.

### Run the Protocol

1. Open the *Run Options* screen.
2. Select the **Full plates** option, if it is not already selected.
3. Enter "4" for the **Number of Full Source Plates**.

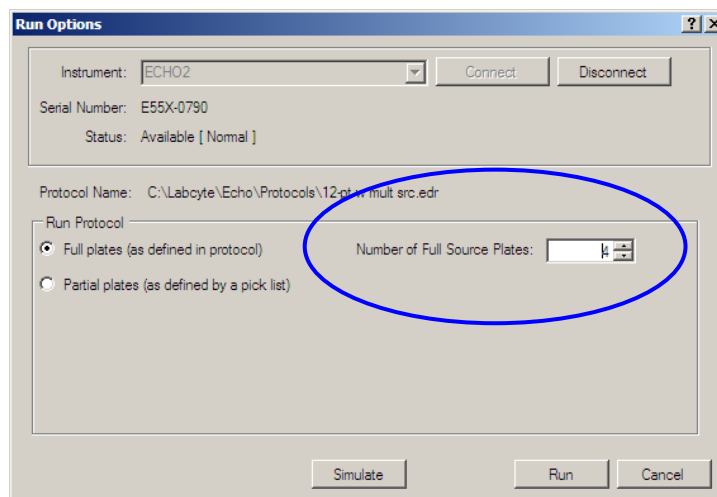
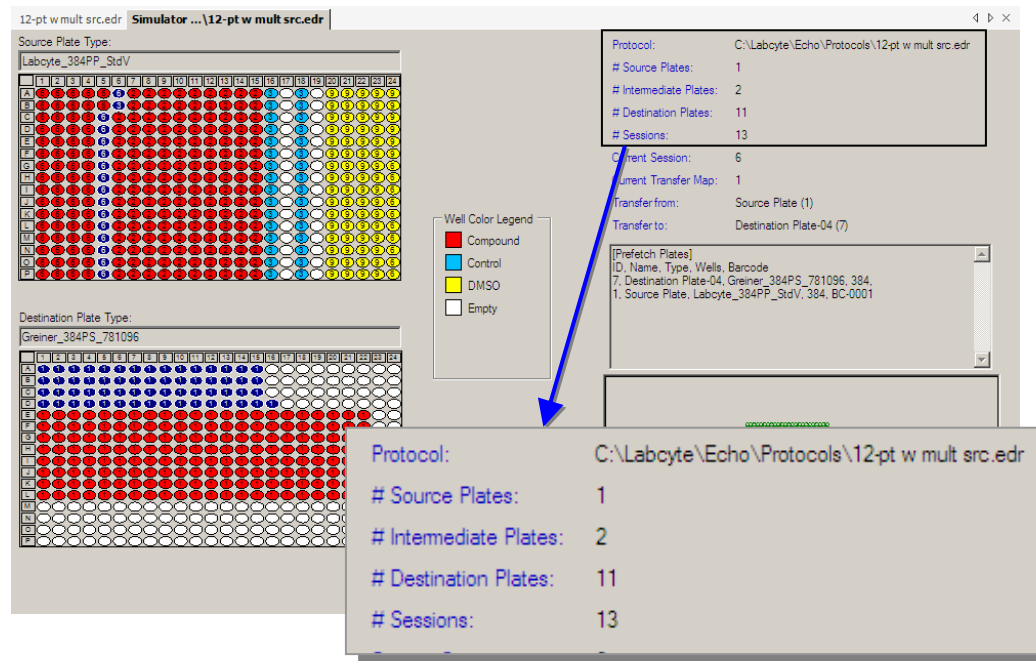


Figure 4-32: Entering number of source plates

- Click **Simulate** to open the simulation window.
- Click **Play** to start the simulation. Since this protocol uses several plates, it requires extra time to load the plate data and start the simulation.



**Figure 4-33: Protocol simulation with multiple source plates**

Once the simulation begins, the software shows the number of intermediate and destination plates that are needed for this run (upper right corner of the screen).

To process 4 source plates, only 5 intermediate plates are needed. If you were to run the protocol 4 times with one source plate per run, you would require 8 intermediate plates. See the following data comparison:

<b>No. of runs</b>	<b>1 run</b>	<b>4 runs</b>	<b>1 run using multiple source plates</b>
# Source Plates:	1	4	4
# Intermediate Plates:	2	8	5
# Destination Plates:	11	44	44
# Sessions:	13	52	49

**Figure 4-34: Plate requirements with one source plate**

Depending on the plate layout of your destination plate (how many empty wells remain after a run), the ability to run multiple source plates in a single run can make use of the empty wells and significantly reduce the number of intermediate and destination plates that are needed.