Organ Bath Systems

ADINSTRUMENTS

Introduction:

The organ bath is a traditional experimental set-up that is commonly used to investigate the physiology and pharmacology of in vitro tissue preparations. Perfused tissues can be maintained for several hours in a temperature controlled organ bath.

After a suitable equilibration period, the researcher can begin experiments. Typical experiments involve the addition of drugs to the organ bath or direct/field stimulation of the tissue. The tissue reacts by contracting/relaxing and an isometric or isotonic transducer is used to measure force or displacement, respectively. From the experimental results dose-response curves are generated (tissue response against drug dosage or stimulus potency).

ADInstruments supply a range of organ baths and transducers for pharmacological research. LabChart software records, displays and analyzes the data which may then be analyzed or exported to graphing programs, such as GraphPad Prism®.

Some of the tissues that may be studied with an organ bath system include:

Smooth Muscle:	**Skeletal Muscle:**	**Cardiac Muscle:**
Vascular, e.g. arterial/aortic rings	Toad Abdominus Rectus	Atrium
Guinea-pig ileum	Chick Biventer Cervis	Ventricle
Vas Deferens (secretory duct of the testicle)	Mammalian Diaphragm	
Uterine		
Colon		

Tissues are usually prepared in a petri-dish containing physiological solution (i.e. Kreb’s solution). The ends of the tissue are then attached to the mounting hook and transducer using silk. It is important to use non-compliant material such as silk or fine wire to mount the tissue.

For tissue ring preparations (i.e. arterial rings), a custom tissue-holder is required (can be sourced by ADInstruments). These may also be made using triangular-shaped wire holders constructed of non-compliant material and secured to the mounting hook on the Panlab tissue-support rod (Figure 1).

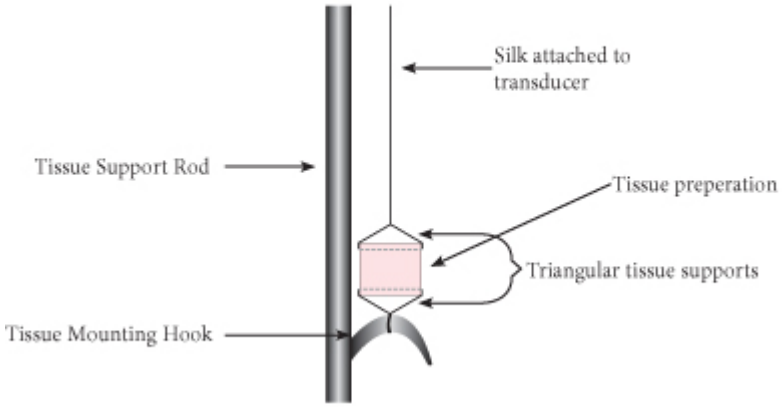


Figure 1. Schematic diagram of a custom-made mounting system for tissue rings.

Panlab Organ Baths:

ADInstruments supply compact and robust, yet versatile organ baths manufactured by Panlab. Each organ bath can take 5, 10, 25 or 50 ml tissue chambers. The tissue chamber can be easily changed by the user and any sized tissue chamber can be used in all the Panlab organ baths. The tissue chambers are automatically filled and emptied using electrovalves with each tissue chamber also having it own gas diffuser which can be regulated.

A heating rod is built into each of the organ bath units and a thermostat controller is included to control the water temperature surrounding the tissue chambers (Figure 2). Pre-heating reservoir coils are also positioned within the water bath to heat the perfusate before entering the tissue chamber. A highly efficient, magnetic water pump is built into the water bath to circulate the heated water and ensure even heat distribution throughout the entire system. Manipulators/micropositioners are supplied for accurate positioning of the transducers and applying pre-tension to the tissue preparations prior to experimentation.

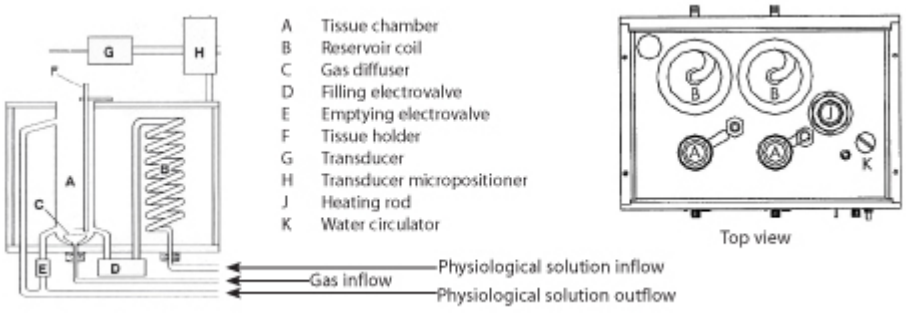


Figure 2. Schematic diagram of a 2 chamber Panlab organ bath.

ADInstruments/Panlab Organ Bath Systems

The Panlab organ baths are supplied as complete, ready-to-use research systems that include all of the necessary hardware and software to perform isolated tissue experiments. Each research system includes:

- An 8 or 16 channel PowerLab data acquisition system
- Multi-chamber Panlab organ baths (4, 8 or 16 chamber systems available)
- Bridge amplifiers (Quad or Octal Bridge Amps)
- Isometric transducers
- LabChart Pro (including Dose Response)

All of these components may be purchased separately, if required.

Single Chamber Organ Bath
ADInstruments supply a single chamber organ bath by Panlab; however, it is only recommended for teaching applications and does not include a thermostat controller.

ADInstruments have a Thermoregulated Water Pump (Model #: MLE2001) that will work with up to four of the single chamber organ baths in series; however, there should be no more than 2 meters distance between the first and last organ baths.

If an external heating system is required, then an "immersion heater/circulator" is suitable (i.e. the TU1 from Thermoline Scientific,www.thermoline.com.au). When used with the Single Chamber Organ Bath, the maximum flow rate of the circulator should not exceed 5 L/min (to avoid excessive pressure within the system). The size of the support clamp, length of the heating rod and intending heating volume should also be checked to ensure they are compatible with the dimensions of the water bath.

Stimulating Electrodes
Electrodes are required if using a stimulator. The platinum electrodes are recommended for most researchers using field stimulation (see table and diagrams below). All of the following stimulating electrodes are supplied with 2 mm plug connections.

Type & Description	Platinum	Stainless Steel
Single Ring (6.5, 8 or 10 mm inner diameter) 20 mm between ring and tissue holder	MLA0301/ID	MLA0305/ID
Double Ring (6.5, 8 or 10 mm inner diameter) 20 mm between rings, 6mm above tissue holder	MLA0302/ID	MLA0306/ID
3 mm Length Pole, 20 mm from tissue holder	MLA0303	MLA0307
Double Ring (6 mm inner diameter) 15 mm between rings, 12 mm from tissue holder	MLA0304	MLA0308

Specify: /ID = 6.5, 8 or 10 mm

Key Benefits

- Available in 2, 4, 8 chamber configuration
- Single water bath provides controlled temperature regulation
- Automated chamber filling and emptying using electrovalves
- All-in-one design provides a compact portable unit

Radnoti Organ Baths:

ADInstruments provide complete 4, 8 and 16 chamber Radnoti tissue-organ bath systems that include a PowerLab, bridge amplifiers, thermo bath/circulator and transducers for the recording of data from isolated tissues.

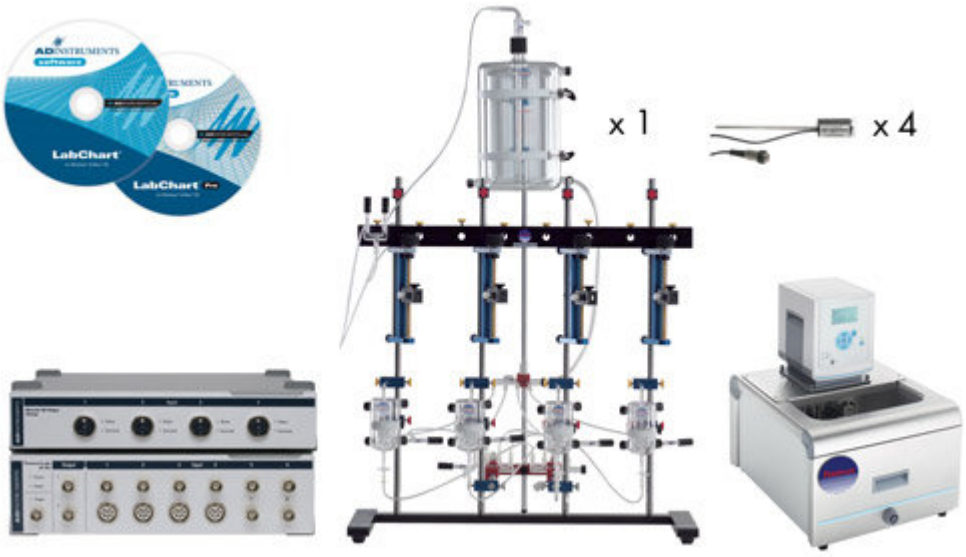


Figure 3. PL3508B60 Radnoti 4 Chamber Tissue-Organ Bath System.

Radnoti organ baths include a single water-jacketed 2 L reservoir for the physiological solution. The solution enters the tissue chamber via a preheating coil that is positioned within the water-jacket of the High-Tech Tissue Organ Bath. The solution in the chamber can be emptied via a drain plug (stopcock) at the bottom of the tissue chamber for a single-pass mode or may be recirculated in a constant flow mode. In the constant flow mode, the solution exits via an overflow outlet positioned at the top of the chamber. Aeration of the physiological solution is provided via needle valves in each bath as well as a frit in the reservoir. Each chamber has its own 5 mg to 25 g isometric transducer (MLT0201/RAD) and transducer positioner.

The modular construction of Radnoti systems permits the researcher to easily interchange tissue chambers and use different sized baths (5, 10, 25, 50, 100, 200, 300 ml). Each organ bath is individually mounted via adjustable ring clamps and is connected to a sturdy steel base that has a resistive coating to avoid corrosion.

Radnoti Thermal Circulator Pump (170051B-V)

This unit incorporates a high wattage heater for rapid warming of the water bath and a two-stage force and suction pump that provides water circulation. Circulation of the water is necessary to maintain a constant temperature in the reservoir and tissue chamber jackets.

When ordering the 170051B-V, please specify the mains power voltage (V) V=115 or 220
Note: *For countries with 115V power service, the Thermo Circulator requies a 20 AMPS outlet.*
For countries with 220V power service, the Thermo Circulator requies a 15 AMPS outlet.

Specifications

- Reservoir Volume: 17 Liters (4.5 Gallons)
- Temperature range: +12 °C to +200 °C
- Temperature Stability: ±0.01 °C
- Maximum Pump Flow Rate: 15 L/min
- Bright, LED digital display
- Stainless steel reservoir with drainage
- Low water level safety shutoff

Figure 4. Thermo Bath Circulator

Each ADInstruments/Radnoti Tissue-Organ Bath System Includes:

- 2L water-jacketed reservoir
- Transducer Positioners
- Oxygenating Bubblers
- Support Stand & Hardware Kit
- Force Transducers (Isometric)
- Cable & Connector Package
- High-Tech Tissue Organ Baths (5-300 ml; User must specify size when ordering)
- Bridge Amplifiers
- Thermo Bath/Heater
- PowerLab Data Acquisition System
- LabChart Pro (including Dose Response)

These systems require manual refilling and emptying of tissue chamber.

Stimulating Electrodes

Radnoti also provides several combination tissue support rods/stimulating electrodes that are suitable for use in organ baths larger than 10 ml. These allow both vertical and horizontal adjustment of the platinum electrodes in relation to the tissue sample to ensure maximal stimulation. A general guide for the use of these electrodes is as follows:

- Tissue strips/spirals: Straight (160152-11), zig-zag (160152-12) or coil (160152-13) electrodes
- Small samples: Coil (160152-13) or flat (160152-15) electrodes

- Blood vessel rings: L-type electrode (160152-14). The tissue ring is slid on the lower support and attached to a tri-shape tissue support (158817 and 158819) and placed under tension between the two horizontal platinum wire electrodes

The above product codes are for combination universal support rods/stimulating electrodes; however, the electrodes may be purchased separately to be interchanged on a universal support rod.

Key Benefits

- Modular components enable tissue chambers to be easily removed and permit investigations with different sized chambers
- Each chamber is individually water-jacketed ensuring uniform circulation and heating
- Reservoir is water-jacketed to maintain solution temperature
- Overflow port allows constant recirculation of perfusate to limit the amount of solution required
- Incorporates Quick Disconnect fittings
- Large range of tissue/organ baths volumes (5,10, 25, 50, 100, 200, 300 ml)

Transducers:

Isometric Transducers

Isometric transducers measure the change in force (in grams [g] or milliNewtons [mN]) produced by muscle relaxation/contraction while the muscle length remains constant. ADInstruments supply the following isometric transducers for use with ADInstruments range of tissue and organ bath models:

- MLT0201 Force Transducer (5 mg to 25 g)
- MLT0201/RAD Force Transducer (5 mg to 25 g) for use with Radnoti Organ Baths
- MLT0202 Sensitive Isometric Force Transducer (0-25 g) ideal for isolated tissue studies and has been designed for operation with ADInstruments range of tissue and organ bath models
- The MLT0210 Teaching Force Transducer is recommended for teaching applications. It operates in the range from 10 mg to 25 g with a resolution of ± 5 mg and is recommended for use with the ML1110 Single Chamber Organ Bath and comes as:
  - MLT0210/D Teaching Force Transducer (MLT0210/D)
  - MLT0210/A Teaching Force Transducer (MLT0210/A)
- MLT0420 is a sensitive isometric force transducer suitable for measuring small forces up to 20 g. The transducer is suitable for connection to ADInstruments Bridge Amps.
- MLTF500/ST (Teaching Force Transducer) A low-cost amplified bridge-type force transducer with measuring range 500 g. The transducer provides a fixed +3 V excitation source for the internal sensor. A range of digital low-pass filters are built-in to reduce signal noise and a software-controlled DC offset adjustment is provided. A detachable mounting bar is incorporated with the unit.
- MLTF050/ST (Teaching Force Transducer) A low-cost amplified bridge-type force transducer with measuring range 50g. The transducer provides a fixed +3V excitation source for the internal sensor. A range of digital low-pass filters are built-in to reduce signal noise and a software-controlled DC offset
  adjustment is provided. A detachable mounting bar is incorporated with the unit.

Isotonic Transducers

Isotonic transducers measure the change in muscle length (in millimeters [mm] or degrees [°] traveled) as it contracts/relaxes against a constant force. ADInstruments supply the following isotonic transducers for use with ADInstruments range of tissue and organ bath models:

- The MLT0015 High-Grade Isotonic Transducer is suitable for the measurement of displacements with preadjusted loads in the range of ±15° (0 to 48 mm displacement)
- The MLT7006 Hall Effect Isotonic Transducer is a low inertia, high performance isotonic transducer with a finely adjustable counterweight suitable for pre-adjusted loads as small as 100 to 200 mg, 0 to 60 mm displacement (± 15°)

Stimulation and Electrode Selection

Stimulation of tissues may be achieved by a number of methods
Field stimulation may be performed using ring or plate electrodes that are placed near (but do not touch) the tissue and have a current passed between them. This method is effective provided that the tissue remains within the field of stimulation and is the most common technique used in organ bath preparations.

A field stimulating electrode is available from Radnoti. It is constructed of platinum wire sealed in glass and designed to insert into Radnoti organ baths. This electrode features a dual vertical spiral coil design with a 1 cm gap that provides a large, uniform electrical field that surrounds the tissue. It is suited for stimulating sections of blood vessels, various other smooth or skeletal muscle preparations or any type of tissue that requires field stimulation and comes with two coil sizes that suitable for different chamber sizes which are:

- 158812 Field Stimulating Platinum Electrode 5-10ml
- 158814 Field Stimulating Platinum Electrode 25-50ml

Alternatively, direct stimulation involves electrode contact with the tissue and is often used to stimulate the tissue via a nerve trunk that innervates it i.e. the isolated diaphragm dissected with the phrenic nerve attached to a stimulating electrode (see Stimulating Electrodes).

Figure 5. Field Stimulating Platinum Electrode

Constant Current versus Constant Voltage Stimulation
For most organ bath experiments, researchers use constant current or constant voltage stimulation. Constant current stimulation is often used as the effectiveness of a stimulus is directly related to the current. That is, current is a measure of the flow of energy into the tissue. The more energy that goes into the tissue, the greater the contraction. An increase in current leads to recruitment of muscle fibers

which contribute to an increase in contractile force. Constant current stimulators are unaffected by changes in load due to factors such as electrode size, contact resistance and polarization.

In constant voltage mode the stimulus voltage is gradually increased until the resultant current (unknown) is enough to induce a tissue response; however, the current itself is not known. A disadvantage of using constant voltage is that polarization of the electrodes may occur in salt solutions (i.e. a build-up on one electrode that increases resistance). The increased resistance between the electrodes means that a greater voltage is required to produce the same current. Polarization of the electrodes may cause the researcher to believe that the tissue is dying when, in fact, the efficiency of the stimulus is reduced.

Stimulators
ADInstruments provide a range of stimulators for organ bath studies. They include the:

- ML155 Stimulator HC is a software-controlled, constant-current stimulator with 100 V compliance designed for use in vitro with isolated nerve, muscle or tissue samples. The unit uses the analog output from the PowerLab to produce isolation and software adjustable constant-current pulses of various durations, frequencies and amplitudes (up to 100 mA).
- ML1001 Electronic Stimulator is designed to provide electrical stimulation in physiological and pharmacological research experiments. It provides options for single pulse, paired pulse, pulse train and repeat pulse operation. Biphasic stimuli are also supported. The stimulator is equipped with a booster amplifier to allow field stimulation with a maximum output of 50V. Input terminals also allow signal mixing and modulation with an external signal. It can provide a constant current and/or voltage stimulation with the ML1101 Stimulus Isolator.
- ML1101 Stimulus Isolator insulates the output for the ML1001 Electronic Stimulator from ground. This minimizes the area of stimulation to ensure greater precision, while reducing stimulation artifacts and facilitating the observation of evoked action potentials. This is not a stand-alone unit and is configured for use with ML1001 Electronic Stimulator.
- The STG4004 and STG4008 Stimulus Generators are multi-channel (4 and 8 channels, respectively) general purpose stimulators, which can be used with PowerLab for data acquisition as well as being controlled by a trigger signal from the PowerLab. For each channel, they can produce an output voltage range between -8 V to +8 V, with a resolution of 1 mV or an output current range between -1.6 mA to +1.6 mA, with a resolution of 100 nA. They are designed to serve a wide variety of applications such *in vitro* and *in vivo* neurophysiology and short-term and long-term stimulations.

Frequently Asked Questions:

What physiological solution should I use?
The solution chosen will have an effect on the viability of the preparation. Solutions such as donor blood may be used, however there are limitations such as availability as well as the possibility of denaturing blood proteins. Researchers would commonly use an artificial plasma-like solution such as Ringer's or Krebs-Henseleit, which contain a buffer to maintain pH levels, nutrients and supply oxygen to the tissue.

What should I use to aerate my physiological solution?

Bicarbonate buffers such as Krebs-Henseleit require aeration with carbon dioxide, therefore, carbogen (95% O_2, 5% CO_2) is commonly used for aerating these artificial solutions. The use of 95% O_2 rather than atmospheric levels of 21% O_2 is necessary to compensate for the lack of hemoglobin or other oxygen carriers in artificial perfusate solutions, ensuring adequate delivery of oxygen to the tissue. If donor blood is used, then a lower O_2 concentration may be used.
In traditional organ bath configurations (using donor blood or a bicarbonate buffer) the tissue is NOT perfused through its blood vessels. Therefore, if the tissue is not normally porous, it relies on diffusion for the delivery of oxygen. The researcher should ensure that the tissue sample is not too thick to avoid its core becoming hypoxic due to inadequate oxygen availability.

What size tissue chamber should I use?
The size of the tissue chamber should be large enough to accommodate the tissue and any additional equipment such as stimulating electrodes. The chamber must have an adequate amount of solution to cover the tissue used. Minimizing the amount of physiological solution in the chamber will conserve the amount of drugs that are required to induce an appropriate response, which may be an important factor.

How long does it take for the tissue to stabilize in the organ bath?
Depending on the tissue type, storage and experimental procedures, the period of equilibration can be from 20 to 60 minutes aft er the tissue has been placed in the organ bath. It is best to consult the latest literature for exact equilibration periods for the tissue being investigated.

There are irregular artifacts in my recording.
The artifacts may be due to vibrations of the silk thread. Make sure the tissue and silk threads are positioned outside of the bubble stream. It is also recommended to have a bubbling rate in which the bubbles, when they reach the liquid surface, do not cause a "boiling" effect at the surface.

IMPORTANT: *Always ensure that the gas diffuser is turned off or has a constant stream of carbogen passing through it before filling the chamber with perfusate. This is necessary to prevent solution entering the diffuser and avoids precipitation of salts which can block the diffuser. Refer to the manual for further details about cleaning and care of the organ baths.*

Glossary:

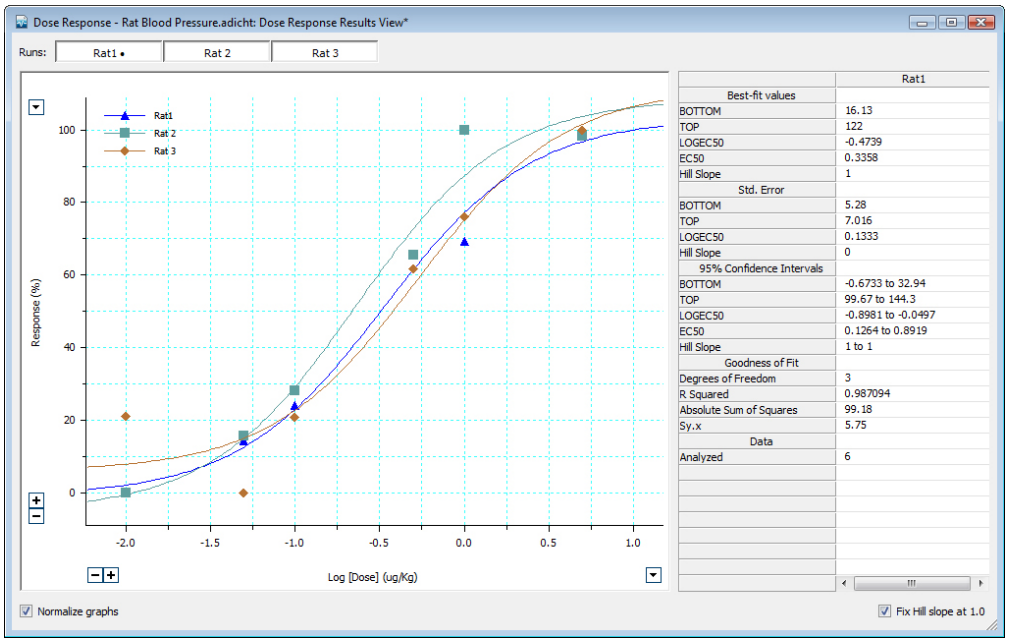
Common Terms in Pharmacology

Affinity: The strength of the reversible interaction between a drug and its receptor, measured by its dissociation constant (Kd).
Potency: The concentration of a drug for a given response magnitude of the biological system. The lower the concentration for a given response, the higher the potency of that compound.
Efficacy: Response magnitude of the biological system upon binding of the drug to its target receptors.
EC50: Half maximal effective concentration of a drug for producing a given effect.
Agonist: A hormone, neurotransmitter or drug that binds to a cell receptor to trigger an active response.
Antagonist: A molecule that blocks a receptor and interferes with or prevents an agonist from eliciting a response.
Clearance: The rate of elimination (removal) of a drug from the body.

Half-Life: The time taken for the concentration of a compound in blood circulation to decrease by 50%.
Tolerance: Reduced effect of an agonist or antagonist following its prolonged administration resulting from the increased metabolism (metabolic tolerance) or decreased receptor sensitivity (pharmaco -or tissue tolerance). Increased dosages are required to produce the desired effect.
Up-regulation: An increase in the number and/or sensitivity of receptors to compensate for the decreased effect of an agonist.

MLS390/7 Dose Response Module for LabChart for Windows:

This Module facilitates analysis of dose response type data from LabChart recordings. The analysis can be done automatically or manually, offline or online. LabChart data recorded prior to the use of the Module can be readily converted for use by Dose Response. The results of the analysis can be exported for use by other applications.



	Rat1
Best-fit values	
BOTTOM	16.13
TOP	122
LOGEC50	-0.4739
EC50	0.3358
Hill Slope	1
Std. Error	
BOTTOM	5.28
TOP	7.016
LOGEC50	0.1333
Hill Slope	0
95% Confidence Intervals	
BOTTOM	-0.6733 to 32.94
TOP	99.67 to 144.3
LOGEC50	-0.8981 to -0.0497
EC50	0.1264 to 0.8919
Hill Slope	1 to 1
Goodness of Fit	
Degrees of Freedom	3
R Squared	0.987094
Absolute Sum of Squares	99.18
Sy.x	5.75
Data	
Analyzed	6

Figure 6. The response points for the three channels and the fitted response curves are displayed in the Dose Response Results View. Fitting parameters for a selected curve are shown in the list on the right.