Technical Bulletin #436 Set Up Guidelines and Dimensional Templates for Fluorescence Plate Readers used with Corning[®] FluoroBlok[™] Insert Systems and Corning BioCoat[™] Multiwell Insert Cell-Based Assays

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CORNING

Please note: unless specified otherwise, the data generated in this Technical Bulletin (TB) used the original (purple) FluoroBlok membrane. We have made improvements to this membrane since it was introduced. The new FluoroBlok uses a black membrane with improved spectral characteristics. General information in this TB applies to both versions of the product, but the specific wavelength ranges mentioned here apply only to the original (purple) version, unless specified otherwise.

For details, see Technical Bulletin **CLS-DL-CC-042**: New PET Membrane for Corning FluoroBlok 3.0 µm and 8.0 µm Pore Size Cell Culture Inserts.

Introduction

Corning FluoroBlok Inserts and Corning BioCoat Multiwell Insert Cell-Based Assays provide platforms for real-time analysis of samples using fluorescence-based detection. These products are used for a variety of applications including analyses of cell motility and compound permeability. To monitor the appearance of fluorescence in the chamber located below the insert, a bottom-reading fluorescence plate reader is required.

This Technical Bulletin describes set-up guidelines for a variety of instruments that are amenable to insert-based assays. To determine the optimal set up parameters, we performed fluorescence-based assays using Corning FluoroBlok inserts in conjunction with a number of fluorescence plate readers including the Applied Biosystems CytoFluor[™] 4000, Bio-Tek Synergy, BMG FLUOstar Galaxy and OPTIMA, PerkinElmer Victor[™], PerkinElmer HTS 7000, Tecan[®] SpectraFluor Plus, and Thermo LabSystems Fluoroskan Ascent.

Important: The information contained within this Technical Bulletin applies only to Corning FluoroBlok Insert Systems and Corning BioCoat Multiwell Insert Cell-Based Assays (i.e., Tumor Invasion Systems, Angiogenesis Systems).

Insert System Assembly and Orientation

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner. With the plate in this position, the Corning logo will be located on the right side.

Plate Reader Set Up

For use with Corning FluoroBlok Inserts, the fluorescence plate reader must support bottom-reading fluorescence detection (i.e., excitation light is presented to the sample through the bottom of the base plate and emitted light is collected from the bottom). If top- and bottom-reading are supported by the instrument in use, one typically may switch between reading modes by software control or manual reconfiguration of the hardware. Before proceeding, ensure that the bottom-reading mode is operative and/or specified by the stored plate reading method (if applicable).

Notes:

- Some systems do not support user entered plate maps or plate definitions. If you are using such a system, the names mentioned for plate choice in this technical bulletin may be subtly different than what is in your software.
- The placement of the insert wells in the 24-Multiwell format is not symmetrical and requires a non-standard 24-well plate dimension.
- In some plate readers the individual 24-well or 24-Multiwell Insert plates must be read without the lid.
- If additional information is needed regarding the reference points and plate reader set up, please contact the instrument manufacturer technical support group.
- Corning is not responsible for damaged property associated with defining new plate maps or instrument modification.

Template Set Up

To add a new plate format template to the plate reader template menu, enter the plate layout dimensions into the plate reader software formula. The required values for some commonly used plate readers are listed in this Technical Bulletin. Detailed drawings with exact well locations are available by contacting Technical Support. Please consult the instrument User Manual to obtain key reference points and units.

Corning Life Sciences strongly recommends you familiarize yourself with the plate reader and have the templates loaded in your plate reader prior to starting your experiment.

Autofluorescence Background

If fluorescence is monitored with a top-reading instrument, the Corning[®] FluoroBlok[™] PET membrane exhibits negligible autofluorescence across the useful range spectrum (490-700 nm). However, a low level of background may be detected with a bottom-reading instrument due to autofluorescence and/or a reflection from the polystyrene base plate. The use of high gain settings ("lamp energy" or other terms may be used) or the lack of appropriate assay controls may promote an autofluorescence effect that is independent of insert-mediated autofluorescence. A gain setting that is too high may also lead to saturation of the detector with samples that exhibit very high fluorescence. The optimal gain or lamp intensity settings must be determined empirically. As a starting point, initiate the experiment with a gain setting or lamp intensity setting at the midpoint.

Fluorescence Detection Issues

Note: Prior to reading Corning FluoroBlok Inserts or Corning BioCoat[™] Multiwell Insert Cell-Based Assays, ensure that the reader has the appropriate Excitation and Emission Filter set installed.

Appropriate Excitation and Emission Filters for detection of fluorophore(s) used in cell labeling must be employed, unless a monochromator-based plate reader (e.g., Tecan[®] Safire) is available. To ensure that all samples are measured as accurately as possible, an appropriate gain or lamp intensity setting must be used.

Applied Biosystems CytoFluor[®] 4000

Note: The CytoFluor[™] 4000 TC (Temperature Control) model cannot read the 24-well or 24-Multiwell formats, as they are too tall to fit in the reader.

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the **Plate Type** list.

Check Installed Filter Set

- Open the CytoFluor software program
- Open the Excitation and Emission Filter drop down menus and choose the appropriate filter set

Note: If the appropriate filters are not installed, refer to the CytoFluor manual for instructions.

Plate Dimensions

DO NOT READ INDIVIDUAL 24-WELL or 24-MULTIWELL FORMATS WITH THE LID ON THE PLATE.

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

- Open the CytoFluor software program
- Open the Plate Type drop down menu and examine the list for the available Corning[®] FluoroBlok[™] Insert Plate format

If the correct plate type is listed, select it and continue. If it is not found, create a new template as follows:

- Select Plate on the menu bar
- Select Define Plate
- Enter the plate type name in the box (e.g., Corning FluoroBlok 24-Multiwell or Corning FluoroBlok 96-Multiwell)
- Enter the appropriate plate parameters for the insert plate format as shown in *Table 1*
- Select Add and OK

Complete Set Up

- Use default settings except as noted below
- Select the Plate Type from the drop down menu
- Select the Excitation and Emission Filter from the drop down menu
- Reads per well 24-well plates: 4
- Reads per well 96-well format: 1
- Center Only
- Gain: 55

Probe Position

The probe must be in the bottom-read position. The probe position is not software switchable; it must be manually repositioned. Repositioning the probe is a brief operation. Please refer to the Applied Biosystems CytoFluor manual for specific instructions.

Powe	Corning FluoroBlok Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert °
ROVVS	4	4	0
Columns	6	6	12
X 1	315	295	309
X 2	2410	2390	2475
Y 1	480	470	420
Y 2	1740	1730	1797

Bio-Tek Synergy

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the **Plate Format** list.

Check Installed Filter Set

- Open the KC4 software program
- Select Wizard
- Select Filter Set
- Select the appropriate Excitation and Emission settings for your fluorophore
- Select Sensitivity

Note: Sensitivity will have to be optimized for your specific application. A setting of 50 is a good starting point. Auto gain is not recommended.

Plate Dimensions

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

- Select System
- Select Plate Formats
- Select appropriate plate type (e.g., Corning[®] FluoroBlok[™] Individual, Corning FluoroBlok 24-Multiwell, or Corning FluoroBlok 96-Multiwell) from the pull down menu

If the list does not contain a plate with the correct dimensions, a new plate can be defined as follows.

- Select New
- Enter the template information in the Plate Description dialog box as shown in *Table 2*
- Select wells to be read
- Click Next until the end
- Click OK
- Save protocol
- Select New for each new plate
- Select read for each new read on the same plate

	Corning FluoroBlok Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Length	127640	127640	127760
Width	85470	85470	85470
Top Left X	14020	12970	14100
Top Left Y	13780	13780	11520
Bottom Right X	110540	109490	113080
Bottom Right Y	71690	71690	74510
Columns	6	6	12
Rows	4	4	8
Well Diameter	6400	6500	3180
Height	23400	24360	19300

BMG FLUOstar Galaxy and OPTIMA

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the **Plate Type** list.

To accommodate the height of the insert plate, spacers are needed to raise the optics above the insert plate surface. To obtain spaces, please call 877-BMG-LABS and request BMG Cat. No. 11-701.

Note: The Galaxy and OPTIMA use different units for entering template data.

Reader Configuration

- Open the FLUOstar software program
- Select the Setup icon on the menu bar to open a drop down menu
- Select Reader Configuration
- Select Fluorescence Intensity and Time Resolved Fluorescence
- Click OK

Note: You will be prompted to check that the right measurement head is installed. The correct head can be identified by the presence of two yellow dots on this surface.

Check Installed Filter Set

- Select the Setup icon on the menu bar to open a drop down menu
- Select Filters
- Examine the list for the appropriate filters. If the appropriate filters are not listed, refer to the FLUOstar manual for instructions.
- Click OK

Plate Dimensions

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

- Select the Setup icon on the menu bar to open a drop down menu
- Select Microplates
- Examine the list for a defined plate with the correct dimensions as shown in *Table 3A (Galaxy) or 3B (OPTIMA)*

If the list does not contain a plate with the correct dimensions, a new plate can be defined as follows.

- Select New
- A new window will open. Enter the dimensions for the insert plate format as shown in *Table 3A (Galaxy) or 3B (OPTIMA)*
- Click OK

Bottom-Read Optics

Turn both optics-positioning wheels so that the first position of each is located at 12 o'clock.

Table 3A – Galaxy

	Corning [®] FluoroBlok™ Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Length	1275	1275	1278
Width	854	854	855
X(1)	140	130	141
Y(1)	138	138	115
X(N)	1105	1095	1131
Y(N)	717	717	745
Format	24	24	96

Table 3B – OPTIMA

	Corning FluoroBlok Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Length	127.50	127.50	127.80
Width	85.40	85.40	85.50
X(1)	14.00	13.00	14.10
Y(1)	13.80	13.80	11.50
X(N)	110.50	109.50	113.10
Y(N)	71.70	71.70	74.50
Format	24	24	96

PerkinElmer Victor™

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the **Plate Type** list.

Check Installed Filter Set

- Open the Victor software program
- Select **Tools** on the menu bar
- Select Filters from the drop down menu. Note: If Filters is grayed out, then select User Level..., then click Advanced.
- Select the Emissions Filters tab and examine the list for the appropriate filter
- Select the CW- Lamp (Excitation) Filters tab and examine for the appropriate filter

Note: If the appropriate filter set is not installed, please refer to the Victor manual for instructions.

Plate Dimensions

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

- Open the Victor software program
- Select **Tools** on the menu bar
- Select Miscellaneous Settings on the drop down menu
- Open the **Plate Types** tab and examine the list for the available Corning[®] FluoroBlok[™] Insert Plate formats

If the correct plate type is on the list, select it and continue. If it is not found, create a new template as follows:

- From the Plate Types Screen, select the Add button
- Enter plate type name (e.g., Corning FluoroBlok 24-Multiwell or Corning FluoroBlok 96-Multiwell)
- Click OK
- On the **Plate Properties** screen, enter the appropriate plate parameters for the insert plate as shown in *Table 4*
- Click OK

Table 4

	Corning FluoroBlok Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Number of Wells in Row	4	4	8
Number of Wells in Column	6	6	12
Unlidded Plate Height	21	22.5	19.05
Plate Height with Cover	23.4	24.4	19.3
Strip Orientation	Horizontal	Horizontal	Horizontal
Edge of Plate to Well Center Horizontal	13.75	12.7	15.38
Edge of Plate to Well Center Vertical	13.75	13.75	11.01
Well to Well Horizontal	19.3	19.3	8.996
Well to Well Vertical	19.3	19.3	8.997

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PerkinElmer Victor[™] (continued)

Entering a Protocol

- Open the Victor software program
- · Select the Protocol Explorer menu bar icon
- Right click the Users Folder
- Select New Protocol Group and enter a name for the new folder. Click OK.
- Right click the new folder and select New Protocol. Enter the protocol name. Click OK.
- Double click the new protocol
- Select the Plate tab and enter the following settings:
 - Measurement height: standard
 - Plate Type: Select appropriate plate (Individual, 24-Multiwell, 96-Multiwell)
- Select the Measurement tab and enter the following settings:
 - Measurement Mode: By plate
 - Click the Labels icon button

If the correct label parameters are already entered, select it and click OK. If not, enter the parameters as follows:

- Select the Fluorometry tab
- Select the Add button
- Enter a new label name (e.g., Corning[®] FluoroBlok[™] 24-Multiwell)
- Click OK
 - On the Fluorometry Label Properties screen enter the following parameters:
 - CW-Lamp Energy Scale slider at mid point
 - CW-Lamp Filter: select from drop down menu
 - Emission Filter: select from drop down menu
 - Emission Aperture: Normal
 - Counter Position: Bottom
 - Counter Height: 0.1
 - Click OK
- Select File on the menu bar and Save the protocol file
- To start a protocol, select **Start**. *Note: If a window with the following error appears:* "*Wallac* 1420 *Exception*. *PLATE ERROR AT MEASUREMENT UNIT*. *The plate is lower than described in the protocol*", *click Ignore*.

PerkinElmer HTS 7000

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the Plate Format list.

Check Installed Filter Set

- Open the **HTSoft** software program
- Select Cancel if the HTSoft Wizard window appears
- Open the Instrument menu tab and select "Parameter Setup..." Select the General tab and under Detection Method, select Fluorescence mode. Select the Measurement tab and check and/or set Excitation and Emission Filters; inspect the drop down menus under Excitation and Emission to examine each list for the appropriate filter set.

Note: If the appropriate filters are not installed, refer to the HTS 7000 manual for instructions.

Plate Dimensions

DO NOT READ INDIVIDUAL 24-WELL or 24-MULTIWELL FORMATS WITH THE LID ON THE PLATE.

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

To see if the plate is already defined:

- Select the **Instrument** tab
- Select Parameter Setup
- Select the General tab
- Browse the Plate Definition file

If the plate definition file is not found, create a new definition as follows:

- Close the Instrument Parameter window
- Under File menu, select HTSoft Wizard
- Select New Plate Definition
- Open the File menu tab and select "Open PlateDef..." and select a plate with similar number of wells. Close the window. Under the File menu, select "Edit PlateDef..."
- Enter the parameters as shown in *Table 5*
- Select the File menu tab, select "Save PlateDef as ... ", and enter the new name
- Select Exit

Reading Samples Using Fluorescence-Based Detection

- Open the HTSoft software program
- Under File, select HTSoft Wizard in the drop down menu
- Select Create and Save a New Method.
- Select Next
- Select the Measurement Parameters tab
- Under the General tab, select the Fluorescence Detection Mode and the Plate Definition, created above
- Under the Measurement tab, select the appropriate Filter settings, and bottom-reading
- · Complete the Method and save with a new method name
- Within HTSoft Wizard, select Run an existing method

	Corning [®] FluoroBlok™ Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Columns	6	6	12
Rows	4	4	8
Well Form	Round	Round	Round
Well Diameter	6.4	6.5	3.18
Upper Left Well	X -265	X -1560	X -450
Start Position	Y 3000	Y 2812	Y 375
Lower Right Well	X 95935	X 94825	X 98895
End Position	Y 60750	Y 60937	Y 63000
Unlidded Plate Height	21000 µm	22500 μm	17020 µm
Plate Height with Cover	23400 µm	24500 μm	19300 µm

Tecan[®] SpectraFluor Plus

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the **Plate Type** list.

Check Installed Filter Set

- Open the Xfluor4.xls software program (enable macros when requested)
- Open the **Xfluor4** menu tab to set all operational parameters (*Table 6*)
- Open the "Edit Measurement Parameter..." tab to check and/or set Excitation and Emission Filters; inspect the drop down menus under Excitation and Emission, and examine each list for the appropriate filter set

Note: If the appropriate filters are not installed, refer to the SpectraFluor Plus manual for instructions.

Plate Dimensions

DO NOT READ INDIVIDUAL 24-WELL or 24-MULTIWELL FORMATS WITH THE LID ON THE PLATE.

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

- Open the Xfluor4.xls software program (enable macros when requested)
- Open the Xfluor4 menu tab. Set all operational parameters (*Table 6*)
- Open the Plate tab (drop down menu) and select "Browse..."to examine the list of available Plate Definition Files (*.pdf). Select the appropriate Corning[®] Insert System plate type.

If the correct plate type is on the list, select it and continue. If it is not found, create a new template as follows:

- Exit Plate Definition File list and return to main Excel screen
- Open the Xfluor4 menu tab again, then select "Edit PlateDefinition ... " tab
- Enter the appropriate plate parameters for the insert plate format as shown in *Table 6*
- Click Update, then click Close (this does not save the file). Under File menu select "Save PlateDef as..." and save the Plate Definition File (e.g., "Corning[®] FluoroBlok[™] 24-Multiwell" or "Corning FluoroBlok Individual").

Reading Samples Using Fluorescence-Based Detection

- Open the Xfluor4.xls software (Enable macros when requested; Connect to reader)
- In the Xfluor4 menu list, open the "Edit Measurement Parameter..." menu item; this opens a tabular listing of available choices
- Under the General tab in the drop down menu, select Fluorescence detection mode
- From the drop down menu, select the appropriate plate definition from the Plate tab; if desired, check the Multiple reads per well box, then select a pattern (e.g., square) and number of replicates (e.g., 2 x 2) from the available options
- Select the appropriate Excitation and Emission filters from the Meas. Params tab (drop down menu); also select Bottom as Read mode, choose a Gain setting method (manual, optimal, or from a specific well); and use default integration parameters (zero time lag, 40 µsec integration time)
- Close the "Edit Measurement Parameter..." menu item, then select the Start Measurement menu item

	Corning FluoroBlok Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Columns	6	6	12
Rows	4	4	8
Well Form	Round	Round	Round
Well Diameter	6.4	6.5	3.18
Upper Left Well	X -265	X -1560	X -450
Start Position	Y 3000	Y 2812	Y 375
Lower Right Well	X 95935	X 94825	X 98895
End Position	Y 60750	Y 60937	Y 63000
Unlidded Plate Height	21000 µm	22500 µm	17020 μm
Plate Height with Cover	23400 µm	24500 µm	19300 µm

Thermo LabSystems Fluoroskan Ascent

Set Up

Prior to reading the insert plate, determine that the reader has the appropriate Excitation and Emission Filter set installed, and the proper insert plate type is specified in the **Plate Format** list.

Check Installed Filter Set

- Open the Ascent software program
- Select the Setup menu heading
- Open the Filters menu heading (drop down list appears)
- Examine the Excitation and Emission Filter combinations, and determine whether the appropriate filters for the fluorophore to be detected have been installed

Note: If the appropriate filters are not installed, refer to the Thermo LabSystems reader's manual for instructions.

Plate Dimensions

DO NOT READ INDIVIDUAL 24-WELL or 24-MULTIWELL FORMATS WITH THE LID ON THE PLATE.

To properly orient the insert plate in the instrument, the well labeled 'A1' should be located at the top left corner.

- Open the Ascent software program
- Select the **Setup** menu heading
- Open the Plate Formats menu heading (drop down list appears)
- Under Setup Plate Templates, select the appropriate template for your application (e.g. "24-well Corning[®] FluoroBlok™" to read Corning FluoroBlok 24-well Individual Insert Systems, "24-Multiwell Corning FluoroBlok" to read 24-Multiwell System, etc.)

Note: The standard pre-set parameters for "24 wells Corning 3047" is similar to that for Corning FluoroBlok Individual Inserts and will work acceptably with the individual insert plates.

If the correct plate map is on the list, select it and continue. To verify the installed settings are correct, do the following:

- Select the Modify box, to view parameters
- Check the parameters against the data in Table 7
- If any parameters are incorrect, edit them and save the edited Plate Format definition. (Template parameter dimensions are in units of 1/10th mm [100 microns])
- Click OK several times to save the new Plate Format and return to the main menu
- Verify the correct parameters were entered and saved

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Thermo LabSystems Fluoroskan Ascent (continued)

If the correct plate map is not listed, you can create a new template as follows:

- Select a similar, but unused plate map. You should select a 24-well template for the 24-Multiwell Insert System, and a 96-well template for the 96-Multiwell Insert System.
- Select the **Duplicate** box
- Rename the template, (e.g., "Corning[®] FluoroBlok[™] 24-wells" to read Corning FluoroBlok 24-well Individual Inserts, or "Corning FluoroBlok 24-Multiwell" to read 24-Multiwell System, etc.)
- Select the Modify box
- Enter the appropriate plate parameters as shown in *Table 7* (Template parameter dimensions are in units of 1/10th mm [100 microns])
- Click OK several times to save the new Plate Format and return to the main menu
- Verify the correct parameters were entered and saved

Table 7

	Corning FluoroBlok Cell Culture Insert	Corning FluoroBlok 24-Multiwell Insert	Corning FluoroBlok 96-Multiwell Insert
Plate Size X	1275	1275	1278
Plate Size Y	854	854	855
Plate Height	210 without lid	225 without lid	193
Well Count X	6	6	12
Well Count Y	4	4	8
Well Diameter X	64	64	31
Well Diameter Y	64	64	31
Well Start X	140	130	141
Well Start Y	138	138	115
Corner Well Distance >	965	965	990
Corner Well Distance Y	′ 579	579	630
Well Type	Circle	Circle	Circle
Can be read with Lid	No	No	No

Note: Gain settings are automatically set by the plate reader.

Corning acquired the BioCoat[™] and FluoroBlok[™] brands. For information, visit www.corning.com/discoverylabware.

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