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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Application Note

Unless specified otherwise, the data generated here used the original (purple) FluoroBlok membrane. We have made improvements to this membrane since it was introduced. The current FluoroBlok uses a black membrane with improved spectral characteristics. General information here applies to both versions of the product, but the specific wavelength ranges mentioned here apply only to the original (purple) version, unless specified otherwise. (Refer to PET Membrane for Corning FluoroBlok 3.0 µm and 8.0 µm Pore Size Cell Culture Inserts CLS-DL-CC-042).

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 application note 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 Bio-Tek Synergy, BMG OPTIMA, Tecan SpectraFluor Plus, and Thermo LabSystems Fluoroskan Ascent.

IMPORTANT: The information contained within applies only to Corning FluoroBlok Insert 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 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. Detailed drawings with exact well locations are available by contacting Scientific 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 auto-fluorescence 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 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.

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 1**
- 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

Table 1. Bio-Tek Synergy

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

BMG 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.

Reader Configuration

- Open the **Optima** 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 Optima 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 2**

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 2**
- Click **OK**

Bottom-read Optics

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

Table 2. BMG OPTIMA

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

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 3)**
- 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 3)**
- 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 3**
- 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**, s**elect **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

Table 3. Tecan SpectraFluor Plus

	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 4**
- 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

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 4** (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 4. Thermo LabSystems Fluoroskan Ascent

	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 X	965	965	990
Corner Well Distance Y	579	579	630
Well Type	Circle	Circle	Circle
Can be read with Lid	No	No	No

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