

Technical Bulletin #436

Set Up Guidelines and Dimensional Templates for Fluorescence Plate Readers Used With BD Falcon™ FluoroBlok™ Insert Systems and BD BioCoat™ Multiwell Insert Cell-Based Assays

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Introduction

BD Falcon™ FluoroBlok™ Inserts and BD 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, BD Biosciences performed fluorescence-based assays using BD Falcon™ 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.

Note: The information contained within this Technical Bulletin applies to all BD Falcon FluoroBlok Insert Systems and BD 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 BD Falcon logo will be located on the right side.

Plate Reader Set Up

For use with BD Falcon™ FluoroBlok Inserts, the fluorescence plate reader must support bottom-reading epifluorescence detection mode (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 can switch between the two 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:

- *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.*
- *BD 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 BD Biosciences Technical Support at 877.232.8995. Please consult the instrument User Manual to obtain key reference points and units.

BD Biosciences 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 BD Falcon™ FluoroBlok™ PET membrane exhibits negligible autofluorescence across the visible spectrum (490-700 nm). However, a low level of background fluorescence is detected with a bottom-reading instrument due to autofluorescence and/or a reflection from the polystyrene base plate. The use of very 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 of 50 or a lamp intensity setting at the midpoint.

Fluorescence Detection Issues

Note: Prior to reading BD Falcon FluoroBlok Inserts or BD 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.

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.

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.

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 BD Falcon 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., BD Falcon™ FluoroBlok™ 24-Multiwell or BD Falcon 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.

Table 1

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon FluoroBlok 96-Multiwell Insert
Rows	4	4	8
Columns	6	6	12
X 1	315	295	309
X 2	2410	2390	2475
Y 1	480	470	420
Y 2	1740	1730	1797

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. Alternatively, the the plate reader can automatically adjust the **Sensitivity** using the **Options** menu.*

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., BD Falcon™ FluoroBlok™ Individual, BD Falcon FluoroBlok 24-Multiwell, or BD Falcon 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

Table 2

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

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, spaces 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

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

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 BD Falcon 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., Falcon FluoroBlok 24-Multiwell or Falcon 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

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

(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., 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
- 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**.*

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**

Table 5

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

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 BD Falcon™ 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., "Falcon FluoroBlok 24-Multiwell" or "Falcon 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

Table 6

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

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 BD Falcon™ FluoroBlok™" to read BD FluoroBlok 24-well Individual Insert Systems, "24-Multiwell BD Falcon FluoroBlok" to read 24-Multiwell System, etc.)

Note: The standard pre-set parameters for "24 wells Falcon 3047" is similar to that for BD 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

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., "BD Falcon FluoroBlok 24-wells" to read BD FluoroBlok 24-well Individual Inserts, or "BD Falcon 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

	BD Falcon™ FluoroBlok™ Cell Culture Insert	BD Falcon FluoroBlok 24-Multiwell Insert	BD Falcon 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

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

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