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SNP TECHNICAL BULLETIN

USING ACYCLOPRIME-FP KITS WITH THE VICTOR

Instrument Setup and Adjusting the "G-factor"

I. INITIAL STEPS

Turn on the Victor and then boot up the computer. Make sure the instrument is recognized by the computer software.

Items in this document that are in the **bold Ariel font** refer to the software used to control the Victor (**Wallac 1420 Manager**). This software should be installed on the computer used to control the Victor by the Service Engineer when the Victor is initially set up. Open the program **Wallac 1420 Manager\Tools\User Level** and set the User Level to **Advanced**. This will allow you to make the necessary software adjustments later.

II. FILTERS NEEDED

Make sure you have received the following filters with your instrument:

DYE	FILTER	CATALOG NO.
R110	**Excitation 480 nm (bandwidth 30)** **Emission. 535 nm (bandwidth 40)**	**1420-5700** **1420-5710**
TAMRA	**Excitation 544 nm (bandwidth 15)** **Emission 595 nm (bandwidth 60)**	**1420-503** **1420-5690**

If any of these filters are missing, contact Technical Support at PerkinElmer Life Sciences (techsupport@perkinelmer.com or techsupport.europe@perkinelmer.com or dial one of the numbers listed in the back of the AcycloPrime kit manual) to obtain the ones you need. If you wish, it is possible to begin work with some alternative filters that may be present, although they may be less than optimal. If you want to do that, discuss the possibilities with Technical Support. If the filters listed in the table have already been installed in your instrument, skip to step IV.

III. FILTER INSTALLATION

1. Defining Filters in Software

Open **Wallac 1420 Manager\Tools\Filters**, and choose **CW-lamp filters\Add**. Enter 480_30 for a name in the box which opens, then click **OK**. Check the box marked **FP** and click **OK** again. Repeat for the 544_15 filter.

Then choose **Emission Filters\Add** and enter 535_40 for a name in the box which opens, then click **OK**. Select the box marked **FP** and click **OK** again. Repeat for the 595_60 filter.

2. Installation of Excitation Filters

Excitation filters are installed in a filter wheel found in a compartment with a blue lid located at the top of the Victor. Move the release lever as shown in the diagram inside the compartment cover and remove the filter wheel from the instrument.

The new filters are installed in open locations on the wheel so that the arrow on the side of the filter points in the direction of the light path (right to left as you face the instrument). If you don't have sufficient open locations, you can remove installed filters that are not needed or use the second filter wheel supplied with the instrument if you have filters of the appropriate dimensions to fit that wheel. Make sure both excitation filters are installed on the same filter wheel.

The filters are held in place with retaining rings. Install the 480_30 filter and the 544_15 filter in available positions using the extra rings that came with the instrument. Make sure to keep track of which filter is installed into which position on the wheel, because you will need the information in item 4 below. Reinstall the filter wheel into the Victor, restore the lever to its original position and close the lid.

3. Installation of Emission Filters

Emission filters are installed in a filter slide located inside a panel on the right side of the instrument. Remove the 3 thumb screws to detach the panel and remove it from the instrument. (If your instrument has a dispenser unit attached, just open the dispenser cover instead.)

In **Wallac 1420 Manager\Tools\Filters**, click the **Filter Slides** tab. Double-click the filter slide being used (usually filter slide A) and click on **Eject Slide**. When the slide emerges from the side of the Victor, remove it from the instrument. Similarly to the excitation filters, install the 535_40 and 595_60 filters into empty positions on the slide. If there are insufficient open positions, you can use filter slide B, provided with the instrument, but make sure that both emission filters are installed on the same filter slide. Again, you must keep track of where each filter is installed for subsequent steps. On the emission filters the arrows should point up.

Reinstall the filter slide by gently pushing it fully into the Victor. It can only be inserted in one orientation. Reattach the side panel using the thumb screws (or close the cover on the dispenser), and press **OK** twice.

4. Telling the software where the filters are located

Assuming the excitation filters are installed in filter wheel A, open **Wallac 1420 Manager\Tools\Filters\Filter Slides** and double-click on **CW-lamp filter wheel A.** If you removed any filters previously installed in the wheel, use the mouse to drag those filters from the wheel diagram to the **Available (unused) Filters** list on the left side of the screen. Drag the 480_30 and 544_15 filters from the **Available (unused) Filters** list on the left into the appropriate openings in the diagram of the filter wheel on the right. When both filters have been installed, click **OK**.

Assuming the emission filters were installed in filter slide A, open **Wallac 1420 Manager\Tools\Filter Slides** and double-click on **Emission filter slide A**. If you removed any filters previously installed in the slide, drag those filters from the slide diagram to the **Available (unused) Filters** list on the left side of the screen. Drag the 535_40 and 595_60 filters from the **Available (unused) Filters** list on the left into the appropriate openings in the diagram of the filter slide on the right. When both filters have been installed, click **OK** and **Close.**

If any of the necessary filters do not appear in the **Available (unused) Filters** list, go to **III.1** and define them.

IV. DEFINING LABELS

In **Wallac 1420 Manager\Tools\Labels\FP**, select **FP-Fluorescein (0.2s)** and **Copy**. Enter **R110** for the name in the box which appears and then click **OK**. Repeat for **TAMRA**.

1. R110

In the list of labels, double-click on **R110**. A box called **Fluorescence Polarization Label Properties** should open.

On this screen you want to define the R110 excitation and emission filters and their positions by wheel or slide and slot location by choosing the filters from the pull-down menu. This table displays arbitrary locations for the filters. Make sure the locations shown in the table reflect the actual physical locations of the filters you installed above. If not repeat step **III.4**. The remaining items should already be as displayed, but if any of them are different, make the appropriate corrections. An adjustment of the G-factor to a corrected value will be made later.

Property	Value
CW-Lamp Energy	**65535**
CW-Lamp Filter	**480_30, Slot A8**
Polarization Aperture	**Normal**
Emission Filter	**535_40, Slot B3**
Emission Aperture	**Normal**
Counting Time	**0.2**
G-factor	**1.0**

Click **OK**.

2. TAMRA

In the list of labels, double-click on **TAMRA**. A box called **Fluorescence Polarization Label Properties** should open.

On this screen you want to define the TAMRA excitation and emission filters and their positions by wheel or slide and slot location by choosing the filters from the pull-down menu. This table displays arbitrary locations for the filters. Make sure the locations shown in the table reflect the actual physical locations of the filters you installed above. If not repeat step **III.4**. The remaining items should already be as displayed, but if any of them are different, make the appropriate corrections. An adjustment of the G-factor to a corrected value will be made later.

Property	Value
CW-Lamp Energy	**65535**
CW-Lamp Filter	**544_15, Slot A4**
Polarization Aperture	**Normal**
Emission Filter	**595_60, Slot B2**
Emission Aperture	**Normal**
Counting Time	**0.2**
G-factor	**1.0**

Click **OK** and **Close**.

V. SETTING UP A FLUORESCENCE POLARIZATION PROTOCOL

In **Wallac 1420 Manager\Tools\Explorer**, open the nested folders **Protocols\Wallac\Fluorescence Polarization**. Copy the locked protocol labeled **FP-Fluorescein (0.2s)**, paste it into a user folder, such as **Wallac 1420 Manager\Tools\Explorer\Protocols\Users\User 1**, and rename the file **R110_TAMRA(0.2s)**. Now when you double-click on **R110_TAMRA(0.2s)**, the protocol will open for editing.

Under the **Measurement** tab, delete **FP-Fluorescein (0.2s)**. Click on the **Label** icon (it looks like a flower), and choose **FP**, then **R110**, and click **OK**. Choose **FP** again, then **TAMRA**, and click **OK**. There should now be two labels under measurement, **R110** first and then **TAMRA**. (To work correctly, PerkinElmer software for allele calling requires that the labels be read in the sequence R110 followed by TAMRA.) The instrument will now measure both dyes when a plate is read using this protocol. **Save** the protocol and **Exit**.

VI. PLATE DEFINITION FILES

Plate definition files for a number of different plates are built into the Victor software (see **Wallac 1420 Manager\Tools\Miscellaneous Settings\Plate Types**). For any 96-well plate not already in the list, it is usually sufficient to use the built-in plate definition file called **Generic 8x12 size plate**. If you are using the generic file or another file already in the list, go to **VI.B.1-2** to set up a plate, then **VI.B.7** and **VII.** to use the plate for determining the G-factors.

A. Creating a Plate Definition File (if necessary)

For a 384-well plate not in the existing list, it may be necessary to create a plate definition file as follows:

From **Wallac 1420 Manager\Tools\Miscellaneous Settings\Plate Types**, click on **Add**, enter your plate type and name, and click **OK**. Measure the plate dimensions or obtain the measurements from the plate vendor and enter the numbers into the table on the screen. As examples, we show measurements that can be used for the MJ Research 384- and 96-well PCR plates.

For a new plate definition, the plate dimensions must be “tuned” using an actual plate. The appropriate G-factors can be determined at the same time. After entering the values for your plate, go to section **VI.B** to “tune” the plate definition.

Sample plate measurements:

Name:	**MJ Research 384**	**MJ Research 96**
Number of well rows:	**16**	**8**
Number of columns:	**24**	**12**
Height of the plate (mm):	**9.1**	**16**
Well locations:		
North:	**12.0**	**14.6**
West:	**8.6**	**11.2**
South:	**4.5**	**9.0**
East:	**4.5**	**9.0**

B. Plate Dimension Tuning Before Adjusting the G-Factor

1. Make a 1:20 dilution in 1X Reaction Buffer of the Terminator Mix from any AcycloPrime kit as follows:

10X Reaction Buffer	10 μL
Terminator Mix	5 μL
ddH_2O	85 μL
Total Volume	100 μL

2. Load 20 μL of this solution into the 4 corner wells of the plate. Centrifuge the plate for 30 seconds to eliminate any air bubbles. Place the plate in the Victor with well A1 at the left rear.

3. Press **Tune**. Click **Yes** to save the new file. The **Plate Dimension Wizard** should be open. Click **Next**. Open the **Fluorescence Polarization** folder, select **R110** (or **TAMRA**) and click **Next**.

4. The program will ask for the scanned area per well. You may use 4.5 (the default), 4.535 for the MJ Research 384-well plate or enter an appropriate value for another type of plate. Then click **Next** to start the measurement of the plate.

5. The instrument reads the 4 corner wells. When it is finished, click **Next**. Select the center of intensity for each of the 4 corner wells by moving the cursor to the center of the red area and clicking.

6. After all 4 centers of intensity are specified, click **Next**. Make adjustments as necessary by referring back to the previous screen and moving the crosshair for a particular well. The software will calibrate the well dimensions and display the adjusted values. Click **Finish**. The new parameters are automatically entered in the **Plate Properties** under **Miscellaneous Settings**. Click **OK** and **Close**.

7. In **Wallac 1420 Manager\Tools\Explorer** open the folders **Protocols\Wallac\Users\User 1,** double-click on **R110_TAMRA(0.2s)**, and click on **Plate**. Choose your plate definition file (e.g., **MJ Research 384**) from the pull-down menu next to **Plate type**. Set **Measure each plate: 1**, then click the button next to **Measurement Height** and enter **5 mm**. **Save** the protocol and close the top two windows.

VII. DETERMINING G-FACTORS

1. From **Wallac 1420 Manager\Tools\Start Wizard**, click **Next**. Locate and select your protocol **R110_TAMRA(0.2s)** and click **Next**. Click-drag the mouse along the letters and numbers at the edges of the plate to define all wells other than the 4 corners as **Empty**. Click **Next**. Then click **Next** and **Finish**. The instrument will read the 4 selected wells in the plate.

2. **Wallac 1420 Manager\Tools\Results of Last Assay Run** displays the data. Export the data to an Excel spreadsheet by clicking on **File\Export**. Save the data under the name **G_factor**. Then open the file in Excel.

3. Separate G-factors must be calculated for R110 and TAMRA using the Victor output data. The output table contains both S and P intensities (in RFU) for both R110 and TAMRA. Using the data for replicate wells, calculate the average RFU for R110 S, R110 P, TAMRA S and TAMRA P. The equation for calculating the G-factor is:

$$G = [S(1000 - mP)] / [P(1000 + mP)]$$

   The diluted Terminator Mix as prepared in **VI.B.1** should give a polarization value of 50 mP for both R110 and TAMRA. Inserting 50 for the mP in the equation gives:

$$G = (950*S) / (1050*P)$$

   Calculate a G-factor for both R110 and TAMRA, using the average S and P values for each dye calculated above. Typical values are between 1.1 and 1.3.

4. Go to **Wallac 1420 Manager\Tools\Labels\FP** and double-click on **R110**. Enter the calculated G-factor and click **OK**. Double-click on **TAMRA** and repeat. Click **Close.**

5. Reread the plate with your protocol as in **VII.1**, but using the new G-factors. The new average mP values for each dye should be near 50 mP. If not, repeat the G-factor determination. Under ordinary conditions, it is not necessary to recalibrate the G-factors. If the reaction conditions, filters, or lamp energy change significantly, or if the instrument is moved to another location, it may be necessary to re-determination the G-factors. G-factors must be determined individually for each instrument.

VIII. USING THE EXCEL TEMPLATES/STACKERS

1. Install the Excel templates in an appropriate location on the hard drive of the computer operating the Victor. For this example, assume this will be a folder called SNPdatafile on drive d.

2. Under **Wallac 1420 Manager\Tools\Explorer\Protocols**, open folders **Users\User 1,** double-click on **R110_TAMRA(0.2s)** and click **Outputs**.

   Check the box next to **File Output** and enter

   File Type: **Excel 5.0** (select from pulldown list)
   FileName: **d:\SNPdatafile\<ProtocolName>_<AssayID>.xls**

   The first line causes the Victor output file to be saved in Excel 5.0 format. The second line describes the path to the folder in which the Victor output file is to be stored and the name to be used to store it. If you use a different location or a different file naming system, adjust the path or other information accordingly.

   NOTE: If the computer does not have Microsoft Excel installed on it, you will not be able to open the data file for inspection if it is saved in Excel 5.0 format. In that case, you may wish to save the output data as a text file instead. Alternatively, you can just copy the Excel file to a floppy for transfer to another computer that does have Excel. On that computer, you can call genotypes by pasting the data file directly into the "generic" versions of the Excel templates from the PerkinElmer website: http://lifesciences.perkinelmer.com/products/snp.asp

   Check all the available choices to include the complete run information in the output file: List, Plate, Protocol, Description, Error description, Notes. If you are using stackers, only the first plate read from the stack will contain the Notes. Since the absence of this field in the output for subsequent plates causes the Excel template to crash, this field is not imported into the template versions 2.0 and higher, but it is saved in the Victor data file for the first plate in the stack.

   NOTE: If you do not have Excel installed on the computer used to operate the Victor, skip to item 4. Otherwise continue.

3. For automatic data dumping into the analysis templates used with stackers:

   Click the **Events** tab

   In the box labeled **Assay End Command**, enter

   c:\Program Files\Microsoft Office\Office\Excel.exe

This is the location of Microsoft Excel. If your computer uses a different location, adjust the path accordingly. **NOTE:** this path is different for Windows XP.

In the next box, enter:

<OutPutFileName> c:\SNPData\SNPmacroVICTOR384v2.0.xls

This is the Excel template to be run. Make sure the version number agrees with the template you are using. If you use the 96 well version, substitute the path and name of the appropriate template: **SNPmacroVICTOR96v2.0.xls**

These commands will save a file with the same name as the Victor output file but with the addition of **_Results** to the name to indicate that the data has been placed into the Excel template for genotype calling. Make sure you are using v2.0 or higher of the appropriate template.

4. Under **Wallac 1420 Manager\Tools\Options**, choose "**Start all assay runs with Start Wizard**".

   If you are using stackers for unattended operation, under **Platemap usage mode**: select **Assay ends after last plate in the plate map** from the pull-down menu. Then make sure that the box that says "**Upon assay completion, a new assay measurement will be performed using the same protocol**" is checked. Click **OK**.

   You must also make sure that your protocol says that there is only 1 plate to read, not the total number in the stack. (The Victor knows when there are plates remaining in the stack). The number of plates to read is displayed at the top of the screen showing the plate display, and can be adjusted under **Wallac 1420 Manager\Tools\Start Wizard.** The third screen allows you to adjust the plate number by clicking the appropriate buttons. It should read "**Plate 1 of 1**".

   These changes ensure that the data from each plate read from the stacker will be stored in a separate file. If the computer has Microsoft Excel installed on it and steps **VIII.1-3** were followed, the data will also be dumped into the Excel template and saved again with a **_Results** suffix. The **_Results** file is not complete until it is examined and the cut-off values are adjusted by the user based on inspection of the scatter plot, but it will already contain the original plate data.