Application Notes for CFX96 Touch™

Instructions for Use with Eurofins GeneScan Kits

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1. INTENDED USE

These instructions are intended for use of Eurofins GeneScan (LR, NR, UMM) kits on Bio-Rad CFX96 Touch™ real-time PCR detection system.

The following instructions are based on Bio-Rad CFX Manager™ software version 3.1.

2. SETUP AND PROGRAMMING OF PLATE AND PROTOCOL TEMPLATE

If you want to use template files for the CFX96 Touch™, a plate and a protocol template need to be programmed and saved.
Alternatively, send an e-mail to kits@eurofins.com informing about your real-time cycler model.

2.1. Bio-Rad Protocol File (.prcl)

For programming the protocol template, Bio-Rad Protocol File (.prcl):

1. Open Bio-Rad CFX Manager™ software and go to “File” and select “New” from the Drop-down menu and “Protocol”.
2. “Sample Volume” is 25 µl
3. For adaptation of the run parameters, select each step and program it as follows by clicking in the corresponding fields (use default ramp rate):
    1. 95.0 °C for 10:00 min
    2. 95.0 °C for 0:15 min
    3. 60.0 °C for 1:30 min
       + Plate Read (camera symbol)
    4. Go to 2, 44 more times
    END
4. Go to “File” and “Save as” to save this Protocol File with a distinctive name.

2.2. Bio-Rad Plate File (.pltd)

For programming the plate template, Bio-Rad Plate File (.pltd):

1. Open Bio-Rad CFX Manager™ software and go to “File” and select “New” from the Drop-down menu and “Plate”.
2. If the Plate Editor opens, go to “Settings”, “Plate Size” and select “96 well” and “BR Clear” under “Plate Type”.
3. For “Scan Mode” select “All Channels” from the menu (a reference dye is not used).
4. Go to “Select Fluorophores” on the right and select the appropriate fluorophore(s) of the kit as described in the kit manual.
5. Go to “Sample Type” on the right side and define respective wells as “Positive Control”, “Negative Control” and “Unknown” for all sample wells.
6. Optional: If you want to use our automated evaluation (Excel™) sheets, the correct detector names/ identifier as mentioned in the respective kit manual, need to be defined as follows:
   Go to “Experiment Settings” and insert new “Target names” (= detector names/ identifier) in the corresponding field and select “Add” to add it to the list and click OK. Select the wells and the suitable

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detector name/ identifier in the drop-down menu of “Target Name”.
7. Go to “File” and “Save as” to save this Plate File with a distinctive name.

Once Plate and Protocol template files are saved, you can access them by going to “File” and “Open” or as described below.

Select your desired Protocol file from your favorite folder. A new window “Run Setup” opens. Select “Plate” from the top flag of this window, go to “Select existing” to open the corresponding Plate template file. If you want to make any changes, go to “Edit selected” on the right and click OK to go back to the “Run Setup” window. If all changes and settings are made, place your plate into the Bio-Rad CFX96 Touch™ RT-PCR machine and go to “Start Run” on the top flag of the “Run Setup” window and start the run.

2.3. Setup for a Quick Start

1. Load plate
2. Open Bio-Rad CFX Manager™ software in the “Startup Wizard” view.
3. Select “Run Setup”.
4. Select Instrument: “CFX96”.
5. Select run type: “PrimePCR” or “User – defined” when using plate and protocol templates (see 2.1 for Setup and Programming of Plate and Protocol Templates).
6. Select “Protocol” from the upper panel and go to “Edit selected” on the right to define the respective PCR temperature and time profile and the sample volume (25 µl).
7. Select “Plate” from the upper panel and go to “Edit selected” on the right to setup the wells as described in the kit manual with well name, respective fluorophores (“All channels”) and plate settings (Plate Size “96 well” and Plate Type “BR Clear”).
8. Click “OK” and then “Next”.
9. Place your plate into the Bio-Rad CFX96 Touch™ machine and start the run by clicking the “Start Run” button in the right corner below.

3. ANALYSIS OF A SAMPLE RUN

1. Open Bio-Rad CFX Manager™ software version 3.1 and select the saved run. Save the resulting Optical file at desired folder.

2. Once the Optical file is opened, go to “Settings” and ensure that the following options are selected:
   Cq Determination Mode = Single Threshold
   Baseline Settings = Baseline Subtracted Curve Fit
   Baseline Settings = Apply Fluorescence Drift Correction

3. The threshold should be placed in the region of exponential amplification across all of the amplification plots. This region is depicted in the log view of the amplification plots as the portion of the plot, which is linear. The threshold line should neither be placed in the plateau phase nor in the initial linear phase of amplification. In general the automatic threshold setting can be used if appropriate.

4. DATA EXPORT

1a. When running the Bio-Rad CFX96 Touch™ without a connected PC, follow these instructions:

- Insert USB into slot on the front of the platform
- Go to “Saved files” folder and select “Real Time Data”
- Select desired file and select “File Options” to export to the USB
- Transfer file to desired location

1b.When running the Bio-Rad CFX96 Touch™ with a connected PC, follow these instructions:

- Go to “Export” tab
- Select “Custom Export” in text format (*.txt) with “Tab” as column separator with the following columns and save it to your designated folder:
  - Well
  - Fluorophore
  - Target Name
  - Content
  - Cq
  - Sample Name
  - End RFU

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5. EVALUATION

Refer to your cycler´s manual for details.
An evaluation (Excel™) sheet can be requested at kits@eurofins.com.

If you use the Eurofins GeneScan evaluation spreadsheet, please follow strictly the spreadsheet instructions of the respective Excel™ sheet.

6. TECHNICAL SUPPORT

If you have any questions or experience any difficulties regarding the use of Eurofins GeneScan products in general, please contact Eurofins GeneScan or your local distributor.

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