Application notes for

ABI 7500/Fast

For Use with Eurofins GeneScan Technologies' Kits

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Cycler application notes
ABI 7500/ FAST

Instructions for Setup and Evaluation

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3. If you use the Eurofins GeneScan Technologies' evaluation spreadsheet, please strictly follow the spreadsheet instructions of the respective Excel™ sheet
4. For Excel™ Evaluation sheets Qualitative/ Quantitative PCR (MACRO versions):
   - Import the Ct and dRn files into the Excel™ Evaluation sheets.
5. For Excel™ Evaluation sheets Qualitative/ Quantitative PCR (NO MACRO versions):
   - Paste all Ct and dRn values from the last cycle in the corresponding columns in the "Ct Report" and view the results in the "Evaluation" panel.

2. TECHNICAL SUPPORT

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

Eurofins GeneScan Technologies GmbH
Engesser Str. 4
79108 Freiburg, Germany
Phone: + 49-(0)761-5038-200
Fax: + 49-(0)761-5038-111
kits@eurofins.com
www.eurofins.com/kits

Instructions for Setup and Evaluation on ABI 7500/ABI 7500 FAST

1. INTENDED USE

These instructions are meant for users of

- Applied Biosystems 7500 FAST

  and

- 7500 Real-Time PCR System.

Eurofins GeneScan Technologies' kits tuned to perform with this Real-Time-PCR-System are

- Low ROX™ (LR) kits

  or

- Universal MasterMix (UMM) kits

The following instructions are based on 7500/ 7500 Fast Real-Time PCR software version 2.0.4 and 7500 Fast System SDS software version 1.5.1.

1.1. Setup and Programming of Plate Documents

To set up a Real-Time PCR analysis with our kits, you may use our run file-templates for ABI 7500 and 7500 FAST.

Please send an e-mail mentioning your cycler model to kits@eurofins.com to receive a copy.

Alternatively, set up your experiment with the following settings:

- For kits that work with a passive reference dye:
  - o select ROX as passive reference dye
- A kit containing the reference dye is e.g.:
  GMO*Screen* RT IPC (LR) 35S/NOS/FMV
  (cat. no.: 5421220301/ 5421220311)
- The kit GMO*Screen* RT IPC (NR) 35S/NOS/FMV
  (cat. no.: 5421220302/ 5421220312)
  does not contain any reference dye.

Please note:

- Always check the manual of the respective kit(s) if reference dye is required.
- Please have the “Passive Reference” option unchecked when running kits not containing the ROX reference dye.

1.1.1. ABI Software 1.5.x

1. Select “Create New Document” in the Quick Startup document window or Select “New” in the “File” menu.
2. Select the following settings:
   * Assay: “Standard Curve (Absolute Quantification)”
   * Container: “96-Well Clear”
   * Template: “Blank Document”
   * Run Mode:
     Select “7500 Standard”, “Fast 7500” or “9600 Emulation” according to the respective manual
3. In the next window, select the “Detector” with the reporters (e.g.: FAM) and e.g.: TAMRA or NONE (for non-fluorescent quencher) as quencher and select ROX as “Passive Reference” if needed, according to the respective kit manual.
4. If you want to use the evaluation (Excel™) sheet, the “Detector Names” of the respective kit manual need to be selected. If the detector names do not exist in the list, select “New Detector” to define name, description, reporter dye and quencher dye according to the manual.

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5. Add them to “Detectors in Document” and click “Next”.
6. Mark all positions on the plate layout with the respective detectors (see kit manual for example of a plate layout) in the “Set up Sample Plate” window.
7. Click “Finish”
8. On the instrument tab set the sample volume to 25 µl below of the “Thermal Cycler Protocol”.
9. Program the Thermocycler Conditions as given in the respective kit´s manual: Select “Standard 7500” or “9600 Emulation” mode as Run Mode and enter the stage for data collection:
10. Save the created file document.
11. If you want to save the file as template, repeat steps 1 – 8 and save it by going to “File” → “Save as” and save the plate document in a respective folder as SDS template file (*.sdt).

    Click “Start” to start the run. The estimated time remaining (hh:mm) is displayed in the upper left corner.

1.1.2. ABI Software 2.0.x

1. From the Home screen select “Advanced Setup” or “Design Wizard” to set up the run. Alternatively you can start directly by clicking on “Template” or “Quick start” to use a previous programmed template or start the run directly.
2. Using Advanced Setup: Under “Experiment Properties” name the experiment accordingly, select “7500 (96 wells)”, Select “Quantitation - standard curve” “TaqMan® Reagents” and “Standard” as ramp speed. Using “Design Wizard”: quantitation method, reagents and ramp speed can be found by selecting the bar “Material and Methods” on the left.
3. Using Advanced Setup: Go to “Plate Setup” to define targets and samples under “Define Targets and Samples”. Select targets and add reporter and quencher dyes/ colours as mentioned in the respective kit manual. Go to “Assign Targets and samples” and select ROX as a passive reference dye on the bottom left.
4. Using “Design Wizard” selects the bars “Targets” and “Samples” (on the left).
5. Go to “Run Method” to set the reaction volume to 25 µL per well and program the Thermocyclers’ conditions as given in the respective kits’ manual.

6. Save the created file document as Experiment document Single files (*.eds) or Experiment Document Template files (*.edt) by clicking on “Save” button in the upper panel (in the “Design Wizard” window go to “Finish Designing Experiment” and click the button “Save Experiment” in the upper left corner).
7. “Start run” by clicking “Run”on the left bar and “Start Run” in the “Run Status” panel.

1.2. Analysis of a sample run and export of analysed data with ABI Software 1.0.x

1. Open the run file with “File” → “Open”
2. Go to “Results” tab and select “Amplification Plot”
3. Mark all wells for further data analysis
4. Select the following settings on the right panel:
   - Data: Delta Rn vs Cycle
   - Detector:
     If more than one detector was used in the run, select the corresponding detector one after the other to define the analysis criteria for each detector.
   - Line Color: Well Color (default setting)
   - Analysis Settings:
     Manual Ct, set the threshold manually or by moving the threshold line with the mouse. 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.
   - Please check the threshold also in the linear view by going to: “Tools” → “Graph Settings” (or right-click → “Graph Settings”) and select “Linear” for the “Y Axis” under “Post Run Settings” and click “OK”. Thresholds are typically in the range 0.03 – 0.1.

     Set the baseline manually from 3 – 15 and click “Analyse” to calculate the data accordingly.
5. For export of Ct values go to “File” → “Export” → “Ct...” (Ct values are exported to a .csv file, delimiter is a comma).

   For export of dRn values go to “File” → “Export” → “Delta Rn...” (dRn values are exported to a .csv file, delimiter is a comma).
   Alternatively you can go to “File” → “Export” →”Results…” to export the values only for selected wells.

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1.3. Analysis of a sample run and export of analysed data with ABI Software 2.0.x

1. Go to “Analysis” on the left bar, mark the required wells for data evaluation and select “Amplification Plot”.
2. Go to “Plot Settings” to adapt the following:
   - Plot Type: dRn vs Cycle
   - Graph Type: Log
   - Color: Well
3. Click on ”Analysis Settings” button in the upper right corner and select “CT settings”
4. Select Baseline Start Cycle 3 and Baseline End Cycle 15 for each target and click on “Apply Analysis Settings”
5. For threshold setting: Go to “option” below the amplification plot window, deselect auto setting threshold and select “show threshold” for each single target.
6. Click on “Reanalyse” in the upper right corner and repeat steps 3 – 4 for all targets.
7. For data export go to “File” → “Export”
8. The “Export Data” window opens. Select “Amplification data” and “Results” under “Export Properties”.
9. Select “Separate Files for Export” from the drop-down menu
10. Enter respective export properties (Export file name and location) and select *.xls as format type.
11. Click the button “Start Export” to export.
12. Go to “Customise Export” and make sure to select “Result” from the “Customise” drop-down menu. Mark all wells in the “Select Results Content” menu for export click the button “Start Export” for export.
13. Finally select “Amplification Data” from the “Customise” drop-down menu. Mark again all wells and click the button “Start Export” for export.
14. If you use the Eurofins GeneScan Technologies evaluation spreadsheet, please follow strictly the spreadsheet instructions.

1.4. EVALUATION

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