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Application Notes for LightCycler® 480 I / II

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 Roche LightCycler® 480 I and II.

The following instructions are based on LightCycler® 480 software version 1.5.0.

Information specific for single kits can be found in the individual manuals for the respective kits. General procedures for set-up and evaluation applicable for all qualitative or quantitative kits are described in these application notes.

2. BEFORE FIRST USE OF A KIT

Please apply color compensation for your individual kit. Consult the kit manual in order to know which dyes are

used in the kit and need to be taken into account for color compensation. The following color compensation kits are available at Eurofins GeneScan:

Cat. No.	Color Compensation Kit
5427200200	Eurofins Color Compensation Kit **FAM, HEX**
5427200201	Eurofins Color Compensation Kit **FAM, R6G**
5427200300	Eurofins Color Compensation Kit **FAM, HEX, Cy5**
5427200301	Eurofins Color Compensation Kit **FAM, JOE, Cy5**
5427200302	Eurofins Color Compensation Kit **FAM, Cy5, R6G**
5427200400	Eurofins Color Compensation Kit **FAM, HEX, Cal610, Cy5**

Important Note

The dye names mentioned in this document are protected trademarks in the US and/or its subsidiaries in certain other countries as follows:
FAM, HEX and JOE are trademarks of Applera Corporation, Cy5 is a trademark of Thermo Fisher Scientific, Cal610 is a trademark of Biosearch Technologies.

3. PROGRAMMING OF THE PLATE DOCUMENT

1. Open LightCycler® 480 software, log in with user name and password.
2. Create a new experiment.
3. In the Setup area of the "Run Protocol" tab, specify the following setup parameters:

   Detection Format: Multi Color hydrolyses probe
   Block Size: 96
   Reaction Volume: 25 µl
4. Activate the required detectors for the respective kit under "Customize": e.g. FAM, HEX and Cy5
5. Use the thermal profile settings given in tables 1 and 2.

Table 1: *Programs used in the thermal profile set up*

Program Name	Cycles	Analysis Mode
Activation	1	None
Amplification	45	Quantification
[Melting Color comp]*	1	Color compensation [Melting curve]
Cooling	1	None

* only if color compensation is done in the same run

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Click on each step to define the following parameters in the "Temperature Targets" panel:

Table 2: *Temperature targets for each thermal profile program, A: activation; B: amplification; C: melting; D: cooling*

A- Activation

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)
95	None	00:10:00	4.4

B- Amplification

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)
95	None	00:00:15	4.4
60	Single	00:01:30	2.2

C- [Melting Color comp] – if color comp. on same plate

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisition (per °C)
95	None	00:00:30	4.4	
40	None	00:00:30	2.2	
65	Continuous		0.03	5

D- Cooling

Target (°C)	Acquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)
40	None	00:00:10	2.2

6. If you run the Color compensation together with samples, click "Subset editor" in the module bar and define a separate sample subset for your Color compensation and name it accordingly (e.g. Color Compensation Kit X).

   6.1 Click "Sample Editor" and select "Color Comp". in "Step 1: Select Workflow" in the upper left Module bar.

   6.2 Define the dominant channel by selection from the Drop down list in the table. (For none fluorescence reactions, select water).

   6.3 Select "Experiment" in the left Module bar and click the button "Start Run" on the right side below. Save it in the respective folder.

4. APPLY COLOR COMPENSATION

Please note:

A Color Compensation experiment has to be carried out only if more than one fluorophore is used.

1. After the run is finished, click “Analysis” in the Module bar on the left side.
2. From the “Create New Analysis” list, select “Color Compensation”.
3. In the “Create New Analysis” dialog, select an analysis subset and a program for the run. For a Color compensation run select:
   - Analysis Type (Color Compensation, default setting)
   - Subset (As named previously e.g. Color Compensation Kit X)
   - Program (Melting Color Comp, default setting)
   - Name (e.g. Color Compensation for Color Compensation, default setting)
4. Click OK to open raw data plots.

5. Click “Calculate” on the Action button area to perform the Color Compensation analysis. The raw data is now compensated as seen on the respective plots beneath the raw data plot. You need to select each Filter Combination by clicking “Filter Comb” button on the right below.
6. Click “Save CC” Object. By default, the CCC folder in your Special Data folder is selected as location.
7. You can now apply the Color Compensation data for the appropriate experiment.

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5. ANALYSIS OF A SAMPLE RUN

5.1 Analysis and export of Cp values

The analysis and export of data from qualitative RT-PCR kits (i.e. GMO*Screen*, GMO*Ident*, DN*Animal*) and quantitative RT-PCR kits (e.g. GMO*Quant*) are identical. The results are evaluated with our Excel Evaluation sheets for qualitative or quantitative tests, respectively (see also Chapter 5 for Evaluation).

1. Open a sample run and go to “Analyses” Modul bar on the left and choose “Overview” from the Drop-down Window panel.
2. From the “Create New Analysis” list, select “Abs Quant/ 2nd Derivative Max”.
3. In the “Create New Analysis” dialog, select an analysis subset and a program. For a sample run select:
   - Analysis Type (Abs Quant/ 2nd Derivative Max, default setting)
   - Subset (All samples or a subset, if defined)
   - Program (Amplification, default setting)
   - Name (e.g. Abs Quant/ 2nd Derivative Max for All Samples, default setting)

4. Click OK to open corresponding amplification curves.
5. First, select the filter combination to be displayed and compensate by using the “Filter Comb” button.
6. To apply Color Compensation to an analysis, select all wells of the plate and click the “Color Comp” arrow-down button. Select between the options “In Use” or “In Database”.
7. Select the Color Compensation object (e.g. Kit X) you want to apply and click OK.
8. The experiment or the analysis charts are redrawn using the compensated data (note that the “Color Comp” button label now says “On”).
9. Click “Calculate”. The curves are then displayed red (positive), green (negative) or blue (uncertain).
10. For export of Cp values, mark all wells of the plate and right-click in the Results table and select “Export Table” to export the data.
11. Select the folder where the data shall be saved and save with File name, Date, Filter and Cp – the information is later needed for the evaluation (Excel™ sheet).
12. Repeat steps 5 – 10 for the other filter combinations.

5.2 Analysis and export of endpoint fluorescence values

1. Go to “Analyses” bar on the top and select “Overview” from the Drop-down menu
2. From the “Create New Analysis” list, select “Endpoint Genotyping”.
3. In the “Create New Analysis” dialog, select an analysis subset and a program in the experiment. For export select:
   - Analysis Type (Endpoint Genotyping, default setting)
   - Subset (All Samples or if a subset was defined)
   - Program (Amplification, default setting)
   - Name (e.g. Endpoint Genotyping for All Samples, default setting)
4. Select two different fluorophores and Click OK to open Endpoint Fluorescence Scatter Plot. If you want to export more than 2 fluorophores (multiplex kits), repeat this step and export the data accordingly.
5. Use the “Color Comp” arrow-down button again to select the Color compensation (“In Use”) or (“In Database”); select the respective file, Click OK and “Calculate”.
6. For export of Cp values, mark all wells of the plate and right-click in the Results table and select “Export Table” to export the data.
7. Select the folder where the data shall be saved and save it with File name, Date and Filter combination – the information is later needed for the evaluation (Excel™) sheets.
8. Repeat steps 4 – 7 for the other filter combinations.

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6. EVALUATION

Refer to your cycler´s manual for more details.
An evaluation (Excel™) sheet can be requested to kits@eurofins.com.
If you use the Eurofins GeneScan evaluation spreadsheet, please strictly follow the spreadsheet instructions of the respective Excel™ sheet.

1. Select module, layout and add additional data like run no., lot numbers, real-time instrument etc.
2. Import the data files from txt file into the Excel Evaluation sheets for Qualitative/ Quantitative PCR (NO MACRO versions), Separator: Tab stop
3. For Evaluation sheet Qualitative PCR: Paste all Cp values and endpoint fluorescence values in the corresponding columns in the “Ct Report” and view the results in the “Evaluation” panel.
4. For Evaluation sheet Quantitative PCR: Paste all Cp values in the corresponding column in the “Ct Report” and view the results in the “Evaluation” panel.
5. NOTE: Cp values ≥ 40 need to be checked again in the LightCycler® 480 Software (sample curves should show typical amplification with log phase).

7. 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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