

Application Notes for Mx3000P™/Mx3005P™

Instructions for Use with Eurofins GeneScan Kits

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TECHNICAL SUPPORT

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

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Instructions for Setup and Evaluation of Eurofins GeneScan Kits on MX3005P™/MX3000P™

1. INTENDED USE

These instructions are meant for users of Stratagene (Agilent) MX3005P™/MX3000P™ for Low Rox (LR) or Universal MasterMix (UMM) Kits manufactured by Eurofins GeneScan.

The following instructions are based on MxPro – Mx3005P software version 4.10.

2. SETUP AND PROGRAMMING OF PLATE DOCUMENTS

For general and more detailed instructions please refer to the user guide of the instrument and respective software version. You may use our template for MX3005P™ (send an e-mail mentioning your cyclor model to kits@eurofins.com) or program your own template with the following settings:

1. Select "Quantitative PCR (Multiple Standards)" on the "New Options" Form.
2. The "Plate setup" screen opens, select all wells and set "Well type" on the right for all wells to "Unknown".
3. Click "File" → "Import Well Names" → "Import Well Names from Excel File" if sample description is desired.
4. Select the respective dyes needed for analysis in "Collect fluorescence data" on the right:
e.g. "FAM", "Cy5" and "ROX"
5. Select a reference dye from the drop-down menu. If no reference dye is needed, select "none".

6. If you use the Eurofins GeneScan GmbH evaluation sheet, we advise you to use the "Assay Names" according to the manual. Click on the button "Assign Assay Names" on the right and select the corresponding assay name. If you cannot find the assay names in the list, select "New" from the drop-down menu to define "Assay" (= identifier/ detector name) and "Assay colour".
7. Click "Next" and edit the "Thermal Profile Setup". Click into the corresponding fields to change times and temperatures according to the instructions in the kit manual. Double-click on "Segment 1" or "Segment 2" to set the number of cycles. For data collection move the loupe symbol with the mouse to the respective cycle.
8. Save the file ("File" → "Save" or "Save as" as *.mxp file and start the run by clicking the button "Start Run" on the lower right side. If you want to save a template of the run, follow steps 1 – 7 and save it in the desired folder with "template" in the file name as a specific template format is not provided in the MxPro software.
9. It is recommended to start the run only when the lamp was warmed-up entirely. The software will remind you if the lamp is not ready.

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3. EVALUATION OF A SAMPLE RUN

1. Click the button "Analysis".
2. Mark all wells for analysis in the analysis selection/ setup tab, then change to the "Results" tab.
3. Make sure that in the "Area to analyze" → "Amplification plots" is selected.
4. Set Fluorescence to "dRn" if a reference dye was selected for normalization (for Low ROX™ kits), select "dR" if no reference dye was selected.
5. Set the threshold for each detector individually either by manual input of a threshold value or by moving the threshold line with the mouse. The threshold should be placed in the region of exponential amplification across all amplification plots. This region is depicted in the log view of the amplification plots as the linear part of the plot. The threshold line should neither be placed in the plateau phase nor in the initial linear phase of amplification. Check the threshold also in the linear view by right-click → "Scale Y Axis" → Select "Linear".
6. Click "Recalc" to adapt the data accordingly.
7. Make sure that "Adaptive Baseline" is selected under "Options" – "Analysis Term Settings" – "Baseline Correction".

4. EXPORT OF EVALUATED DATA

1. Select "Text report" in the "Area to analyze".
2. Select the following columns for data export:
 - Well
 - Well Name
 - Dye
 - Assay
 - Well Type
 - dR Last or dRn Last
 - Threshold (dR or dRn)
 - Ct (dR or dRn)
3. a) For evaluation in your personal spreadsheet: Right mouse click → "Export Text Report" → "Export Text Report to Excel". Copy Ct and dRn/dR Last values from Excel™ file to your evaluation sheet.
3. b) If you use the Eurofins GeneScan (Excel™) evaluation spreadsheet, please follow strictly the spreadsheet instructions: Right mouse click → "Export Text Report" → "Export Text Report to Text File" and save the generated Text file (*.txt) in your desired location. Import the data if you use the Eurofins GeneScan automated evaluation sheet.

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