



Instructions

General

Copy data from PCR software

Export the data from the PCR run to Excel® if the PCR instrument software offers this opportunity or copy the data directly to the Calculation Template sheet "Instrument Data" and sort the data as necessary. **Convert all negative values to an " " (empty field) and for the German version of Excel, the number format from "point" to "comma"** (e.g. 30.02 to 30,02).

Step 1

Enter standard curve data

Perform a standard curve run at least once for every lot of the **foodproof® Allergen Detection Kit**. Please follow the instructions in the Quick Guide. Copy Cq values in the FAM channel of both replicates of the standard curve to the Calculation Template sheet "Quantification". Enter the data into the upper orange fields to calculate the standard curve.

Note: Saved standard curve data are only valid for re-use for runs performed on the same instrument! For subsequent runs with the same lot of the PCR Kit, it is possible to transfer the standard curve data from other runs performed with this lot. Only the undiluted Quantification Standard (Calibrator) and a Negative Control in the new PCR run will be necessary in this case.

Step 2

Enter control sample data

A positive and a negative control must be performed with each PCR run.

The Negative Control (NC) serves as contamination control in the FAM channel. The Internal Amplification Control (IC) in the HEX channel should not be inhibited.

A Positive Control DNA is provided with the Allergen Detection Kit. For Quantification, use the undiluted Standard (Calibrator) DNA of the RM 800.

In a standard curve run, copy the data from one of the replicates. For runs that just use a Calibrator, copy the Cq values in the FAM and HEX channels to the Calculation Template sheet "Quantification". Enter the data into the lower orange fields. In a standard curve run, copy the data from the replicates to the Calculation Template sheet "Quantification". Enter the data to the lower orange fields.

Note: If "passed" is displayed next to the sample, no issues are present with the control samples. If "invalid" is displayed, PCR data should not be analyzed further.

Make sure that there is no sample mix up, and data generated by the Negative Control and Standard were entered correctly. If the warning "invalid" still persists, contamination occurred during the experiment (Negative Control) or a wrong sample type was chosen for the calibrator. In this case, a valid quantification cannot be conducted and the PCR run needs to be repeated.



Calculation Template
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Step 3

Enter sample information

In the green fields of the Calculation Template sheet "Quantification", enter the sample ID or name for each sample, the PCR position (well) and specify the "weighed sample amount" and the amount of PCR template "DNA-extract used for PCR".

Step 4

Enter sample data

For each sample, copy the Cq values in the FAM and HEX channels from your real-time PCR instrument software to the Calculation Template sheet "Quantification". Enter the data into the green fields.

Note: Make sure there is no mix-up of channels, sample data and sample information in the transfer.

Pay attention to the "Comment" field; e.g., when "invalid" is displayed, PCR data should not be analyzed further. If the IC result shows "invalid", sample inhibition occurred.

Abbreviations

Cq Quantification Cycles (synonyms: Ct (cycle threshold) or Cp (crossing point))

PCR Kit Lot: Please enter SAMPLE data in green fields!PCR Kit Lot: [illegible]