

Analysis with “QPCR analysis template.xls”

If you choose to do your calculations without the aid of SDS2.3 you will still have to use SDS2.3 to export the individual Ct values as follows:

1. AnalysisAnalysis Settings
2. In the Ct Analysis Section of the Analysis settings Window:
 - from the “Detector:” drop down menu select “All Detectors”
 - Choose “Automatic Ct” (this will also default you to “Automatic Baseline”). We recommend that you use the automated Ct and baseline features for optimum data quality, but you can also choose to set each of these manually.
3. Left click Apply then left click **OK**.
4. AnalysisAnalyze
5. FileExport:
 - From the “Export:” drop down menu, select “Results Table”.
 - In the “From:” section check “All wells” to export data from all the wells containing data of “Selected Wells” to export data from highlighted wells only.
 - In the “Format:” section select SDS2.3.
 - Give the file a proper name and make sure the “Files of type:” drop down menu is set to “Tab-delimited Text(*.txt)”.
 - Left click the **Export** button.

We recommend importing the data into MS Excel before further use. The data can be copied and pasted or the text file itself can be opened with (and saved as) a MS Excel file. From this exported file you should have the individual Ct values. These values can be copied and pasted as appropriate into the “QPCR analysis template.xls”. This spreadsheet will automatically calculate the RQ as well as the fold change values. The derivation and explanation of the formulas used in this sheet are explained in ABI User Bulletin#2.

¹ *The calibrator sample serves as the basis for the comparative results as gene expression levels in samples are calculated relative to gene expression levels in the calibrator sample.*

² *Endogenous control targets are typically constitutive RNA or DNA sequences that are present at a statistically consistent level in all experimental samples. By using the Endogenous control as an active reference, the data from the amplification of the targets can be normalized for differences in the amount of total nucleic acid added to each reaction*