

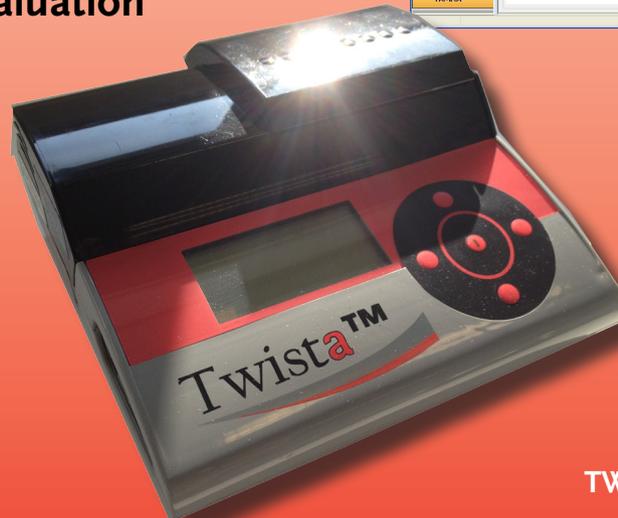
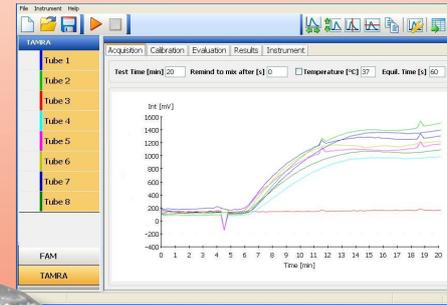


TwistDx

Twista® Studio

Software

Quick Guide to Data Validation and Evaluation



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1 Introduction

This guide is intended to help the first-time user to become familiarized with the validation and evaluation functions of the Twista® Studio software. The guide should be used in conjunction with the example data supplied. **Note that there are two example datasets**, which differ only in the decimal point system as setup on your computer. If you use a point as decimal separator, use the dataset 'example data EN': If you use a comma as decimal separator, use the dataset 'example data DE'

This guide assumes that the Twista® Studio software has been setup on your computer. The guide will take the user sequentially through the various validation and evaluation techniques, showing how the sample results change depending on the parameters used. At the end, the user should have attained a working understanding of how the software should be used.

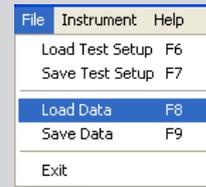
2 Getting Started

Start the Twista® Studio software by double clicking on the Twista® Studio icon which appears on the desktop:

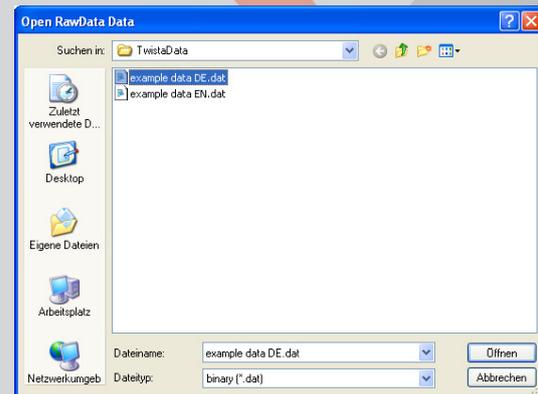


The Twista® Studio software will start.

Click the File menu then select 'Load Data':



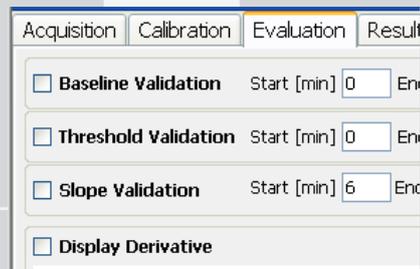
Select the example data file you require from the dialog which appears (which dataset is selected depends on your decimal point system, see left):



Note that the dataset supplied has data in the FAM channel only: The TAMRA channel is empty. As first step, we need to clear all validation and evaluation options. Select the TAMRA channel by clicking on its button on the bottom left of the screen:



Then ensure that all of the validation and evaluation options are cleared by first clicking the 'Evaluation' tab and then deselecting any that may be selected, so that the options appear thus:



We will work only with the FAM channel, so select this by clicking on the FAM button on the bottom left of the screen:



You will notice that the FAM data will appear on the main graphic on the evaluation page.

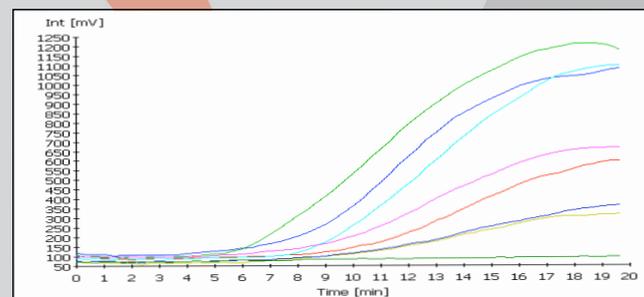
3 Baseline Validation

The first test criterion applied to the sample data is the baseline validity check. During the early phase of amplification, there should not be enough signal, even for a positive sample, to be detected by the system. During this time, the baseline should remain stable, and should not be too high. The result of this check will be that samples will be deemed Valid or Invalid.

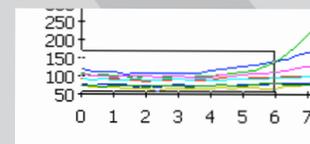
Observe the data in the main graphic (if a table is displayed, click on the Display as Graphic/Table button in the toolbar:



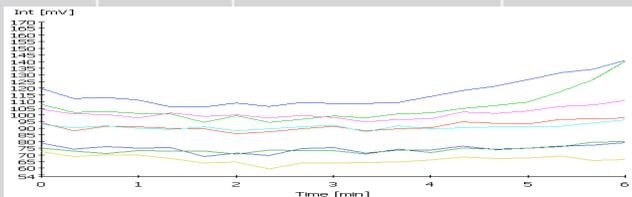
Ensure that the 'Display Derivative' option is deselected: We want to observe the data in normal mode. The data should appear thus:



As can be seen, the signal traces for all samples are reasonably constant for the first 4 minutes. We want to observe this part of the data more closely, so zoom into the region by clicking the mouse and dragging the zoom-box as shown right:



The data will now be displayed with the selected zoom range:



As can be seen, the blue trace has already started to increase significantly around 4 minutes. For this reason, we should restrict the baseline validation check to the time up to 4 minutes. To see the effect of a sample being invalid, enter lower and upper limits which will obviously exclude that sample by analyzing the data. A lower limit of 85, for example, should invalidate the bottom three traces in the data. Setup the baseline validation parameters as follows, then select the baseline validation checkbox:

Baseline Validation Start [min] 0 End [min] 4 Lower Limit [mV] 80 Upper Limit [mV] 300

You will see this reflected in the sample panel to the left of the screen, note how tubes 6, 7 and 8 are now deemed invalid (shown right) :



Enter a reasonable lower limit value (for example 50) and re-select the baseline Validation checkbox: (you will note that if any parameters are changed, the checkbox is automatically deselected) With this new lower limit, all samples will become valid.

Try changing the upper limit by observing the data. Also use an end time which is clearly too high, for example with upper limit of 120, all samples are valid for a 5 minute end time. Change the end time to 7 minutes, and tubes 1 and 2 become invalid. A good suggested parameter set for the data as displayed would be:

Baseline Validation Start [min] 0 End [min] 5 Lower Limit [mV] 50 Upper Limit [mV] 130

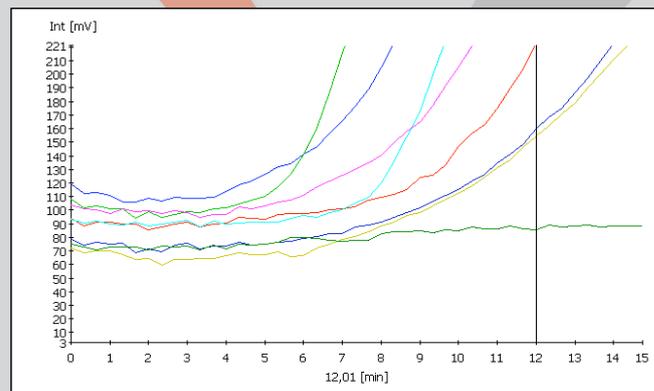
Now that we have created a reasonable baseline validation check, we can proceed to the data evaluation checks.

4 Threshold Validation

Sample validation using the threshold function involves analyzing the data over a period of time to check whether the signal exceeds the mean initial background noise level by a user-determined amount.

The time range used should cover the initial part of the reaction, before the signal starts to increase.

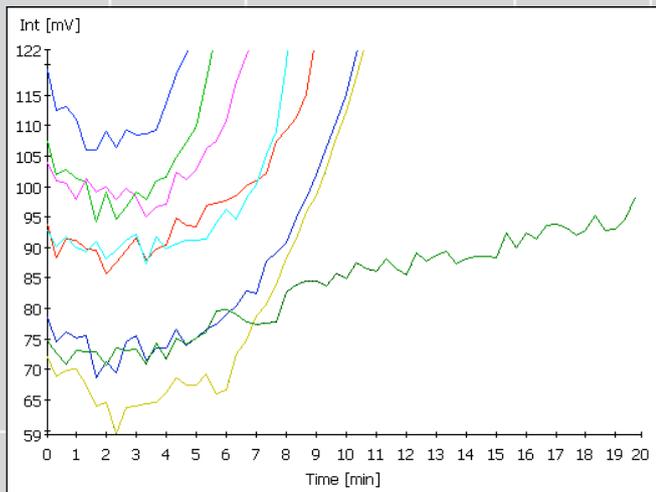
Zoom into the data again, to observe the low intensity signal region:



As can be seen, after 4 minutes, sample 1 has started to rise significantly. Enter threshold values as below:

Threshold Validation Start [min] 0 End [min] 4 Threshold(*SD) 3 Validat. Time [min] 1

Note; that this causes all samples to be identified as positive, including the negative control sample. To determine the cause of this, zoom into the data to look closer at the sample in tube 8:



As can be seen, the negative sample shows a slight slope. In order to allow for this, the end time must be long enough to incorporate the slight increase in sample 8. Setup parameters as follows:

Threshold Validation Start [min] End [min] Threshold(*SD) Validat. Time [min]

The sample panel to the left of the screen should now show sample 8 negative, all other samples positive.

5 Slope Validation

The Slope Validation test is used to identify that the slope of the signal has risen sufficiently over a user-determined level to be deemed as positive. For setting up this evaluation, it is useful to view the data in Derivative mode, which displays the slopes of the signals. Select the Display Derivative option:

Display Derivative

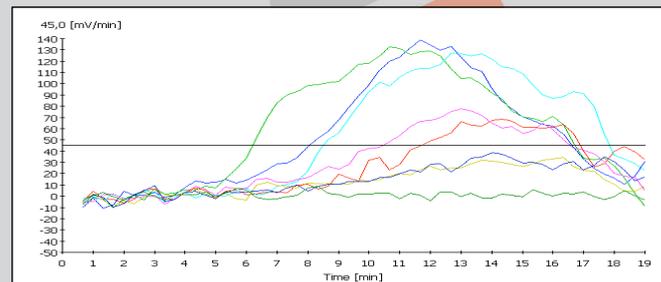
Then activate the horizontal cursor (this is also very useful for evaluating the data) by clicking on the horizontal cursor icon in the toolbar:



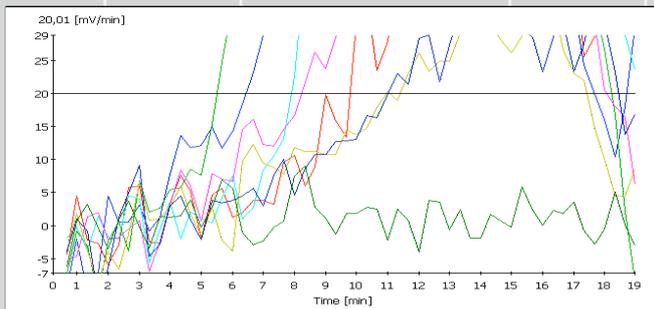
Expand the ordinate axis by clicking on the Auto X expand icon in the toolbar:



The data should now be displayed thus:



Click the mouse on the horizontal cursor and drag it up or down. Find the point where the ordinate value clearly differentiates positive samples from the negative sample (lowest, green trace on the graphic, representing sample 8). You may need to zoom into the data further to be able to see this clearly:



As can be seen, a slope value of 20 clearly differentiates the negative and positive samples, and also gives some headroom to avoid false positives (If we had chosen a slope of 10, for example, then subsequent negative samples may have exceeded this threshold).

Enter the following values into the Slope Validation options:

Slope Validation Start [min] 1 End [min] 19 Period [Points] 3 Slope [mV/min] 20

Note that these parameters produce correct identification of sample 8 as negative, all others as positive.

6 Interpreting the Results tables

The final result of all validation checks is shown in the results page. Click the Results tab:



The table at the top of the page shows the combined results of all tests. This appears as follows:

Final Outcome Test							
Tube1	Tube2	Tube3	Tube4	Tube5	Tube6	Tube7	Tube8
Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative

As can be seen, sample 8 is negative, all others are positive. Note that this table combines results from both channels (in this example FAM and TAMRA). The second table shows individual channel results, and appears as follows:

Result Details								
Tube1	Tube2	Tube3	Tube4	Tube5	Tube6	Tube7	Tube8	Info
Valid	Valid	FAM Baseline						
Positive(12,0)	Positive(12,7)	Positive(12,0)	Positive(12,0)	Positive(12,0)	Positive(12,3)	Positive(12,0)	Negative	FAM Threshold
Positive(7,0)	Positive(6,0)	Positive(10,3)	Positive(8,3)	Positive(6,7)	Positive(11,3)	Positive(11,7)	Negative	FAM Slope
Positive	Negative	FAM						
								TAMRA Baseline
								TAMRA Threshold
								TAMRA Slope
								TAMRA

As the dataset had no TAMRA data and was thus deselected, the bottom half of the table is empty.

The top half of the table shows the result for each individual test, i.e. Baseline, Threshold and Slope evaluations, and the combined result for this channel. Note the figures in brackets after threshold and slope evaluation result data: these represent the time at which the sample was first deemed positive.

For a description of the test criteria, please refer to the Twista® Studio Manual.



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