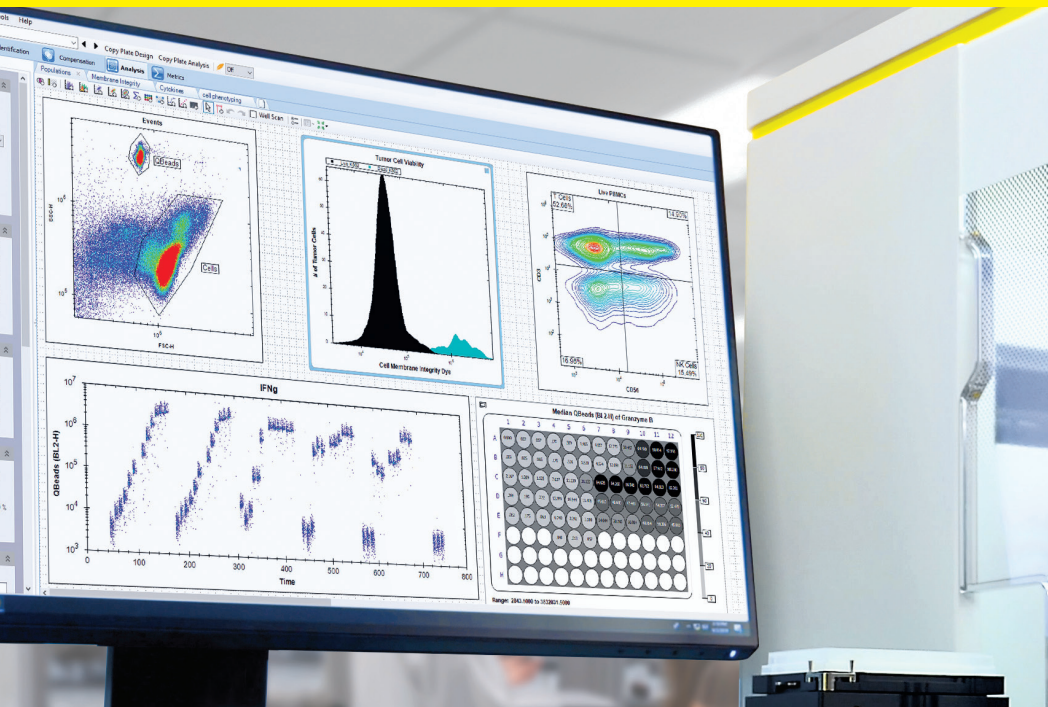


iQue® Signal Reduction



User Guide

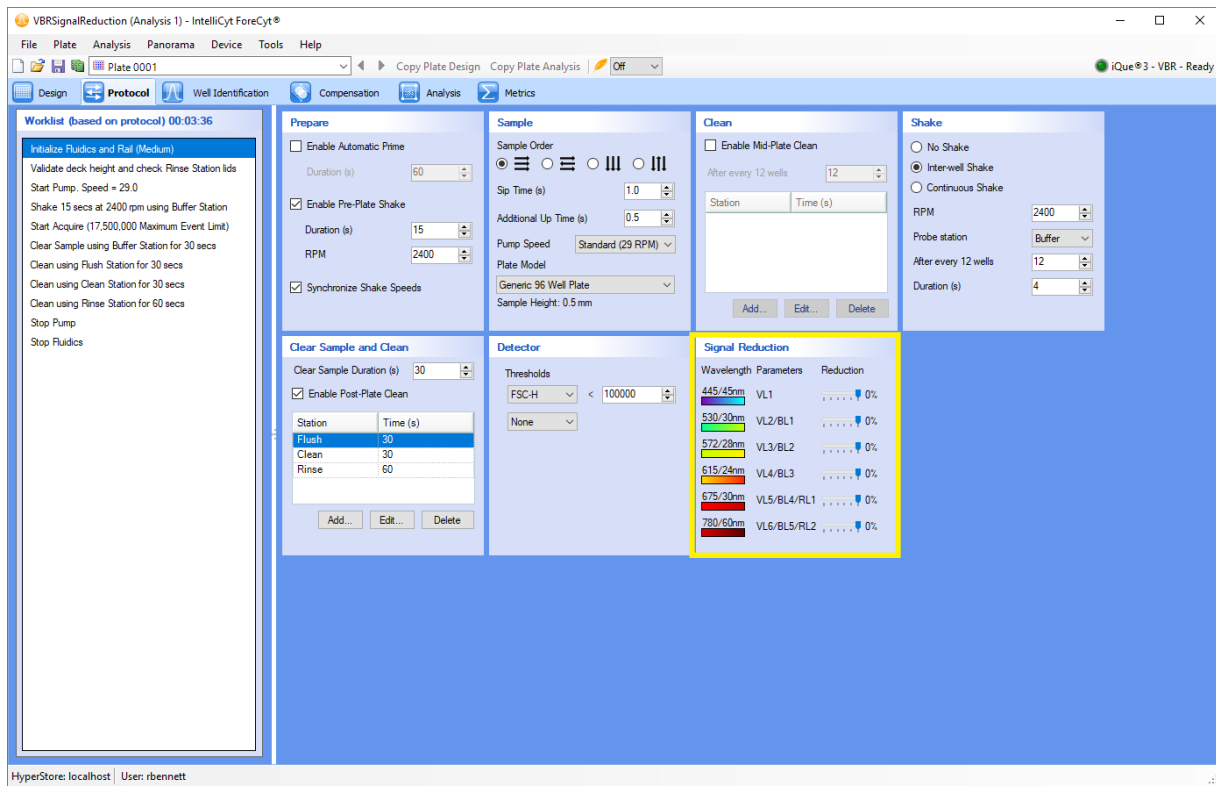
The iQue® Signal Reduction module is a licensable feature in the iQue Forecyt® software which allows users to decrease the signal for selected acquisition channels. Therefore, signals that were previously too bright can now be easily visualized. The feature is activated through the protocol setting for all iQue® Screener PLUS and iQue® 3 instruments.

Background

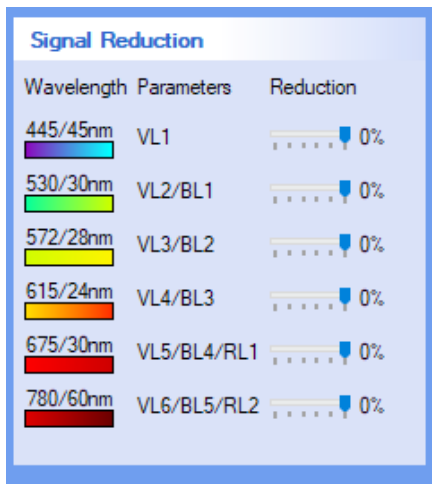
The Signal Reduction module allows users to reduce the signal for selected channels permitting better visualization of samples at the upper limit of detection. This feature is activated as part of the experimental protocol and will only remain in effect for the duration of the run. Once the run is completed all settings will automatically return to default settings with no Signal Reduction applied. This prevents changing system values for other experiments unintentionally. If a user wishes to run the same protocol with Signal Reduction applied the values can be saved as a template and applied to future runs.

Signal Reduction Protocol

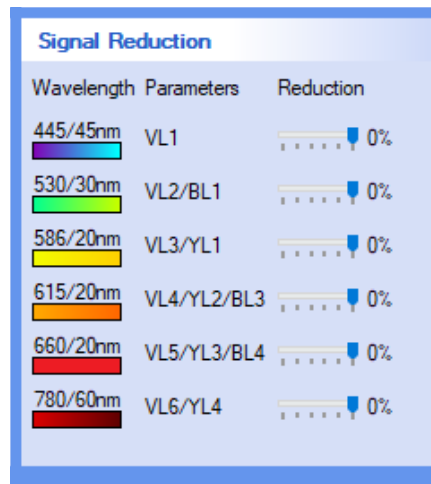
Once licensed, a new "Signal Reduction" section will be added to the standard experiment protocol.



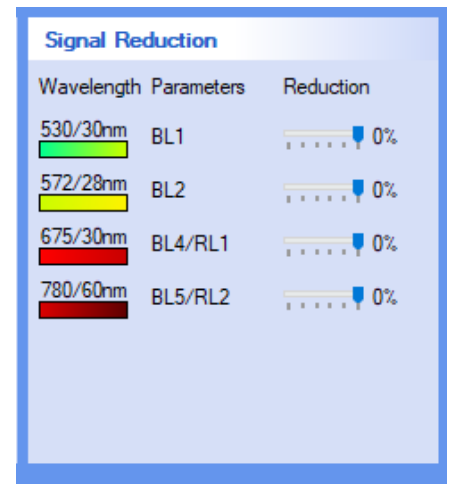
Each licensed instrument will have a Signal Reduction section at the end of the protocol that is specific to the channels of that instrument. This will show the channels that are able to be reduced, the parameters affected, and the amount of reduction as a percentage of the baseline signal. The percentage sliders will all be zero by default and can be adjusted between 0% and 100%. The following figure shows the protocol settings for each instrument:



VBR Signal Reduction

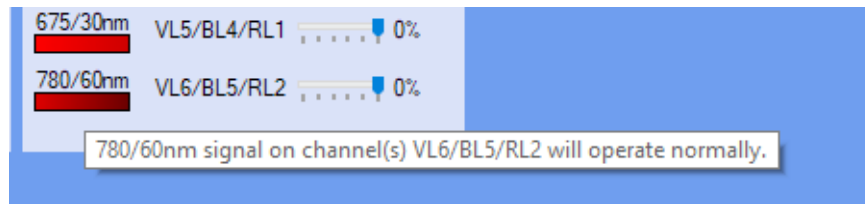
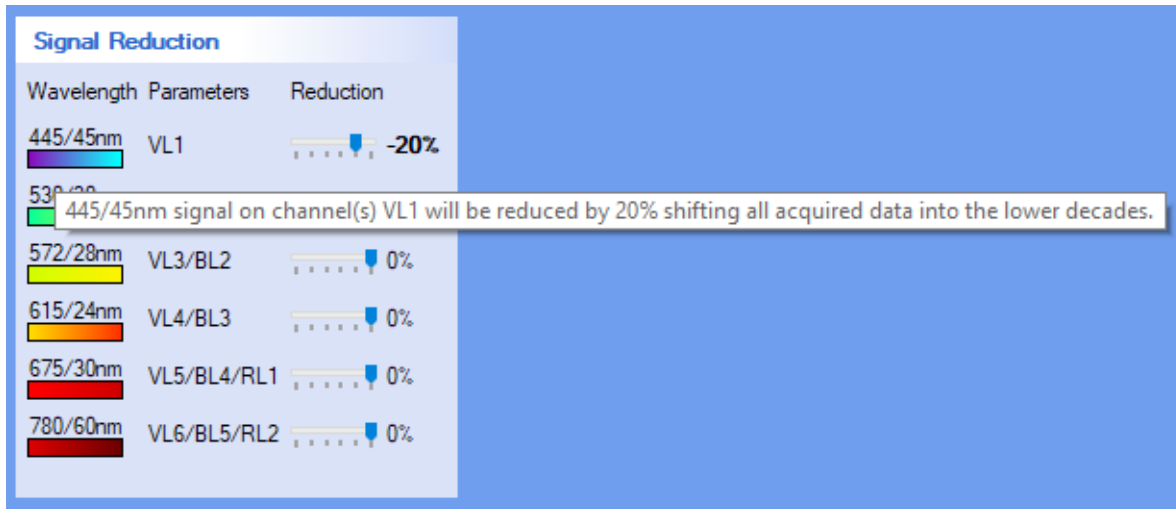


VYB Signal Reduction



BR Signal Reduction

When Signal Reduction is active, the percentages in the protocol will be bolded. Hovering the mouse over the signals being reduced will show a tooltip describing the effect of the reduction.



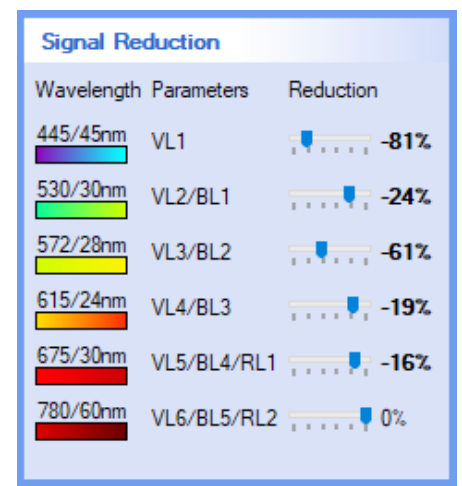
A tooltip is also displayed for channels not being reduced.

Signal Reduction by Wavelength/PMT

When Signal Reduction is applied each channel on that PMT will be reduced. For example, if you apply signal reduction of 20% for the 780/60nm wavelength for a VBR then the VL6, BL5 and RL2 channels will all be reduced by 20%.

Granularity

The Signal Reduction adjustment is percentage based. You can adjust the percentage in 1% increments. The large moves (clicking on track or using page up/down keys) will be 20% increments and the small moves (using arrow keys or slider) will be 1%.



Percentage of Baseline PMT Voltage

Signal Reduction is applied as a percentage. The percentage of is from 0% to 100% between the baseline PMT voltage of the instrument and the minimum allowed voltage of the PMT.

0% = No signal reduction, data is acquired using baseline PMT Voltage

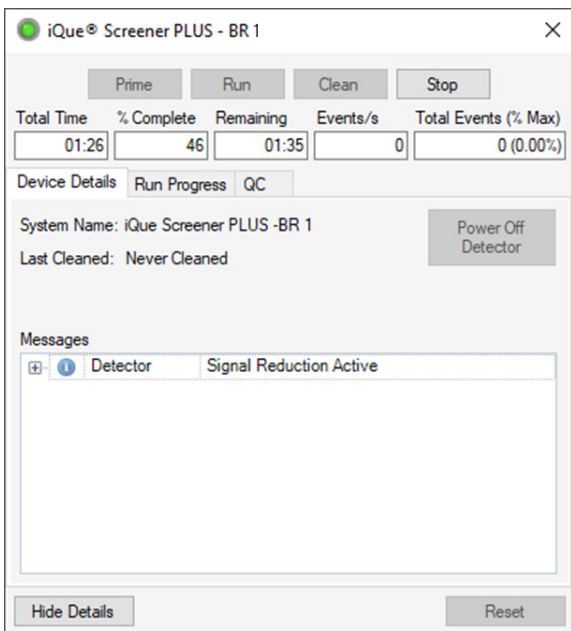
100% = Maximum reduction, data is acquired using lowest PMT Voltage allowed on the cytometer

Protocol Specific Acquisition

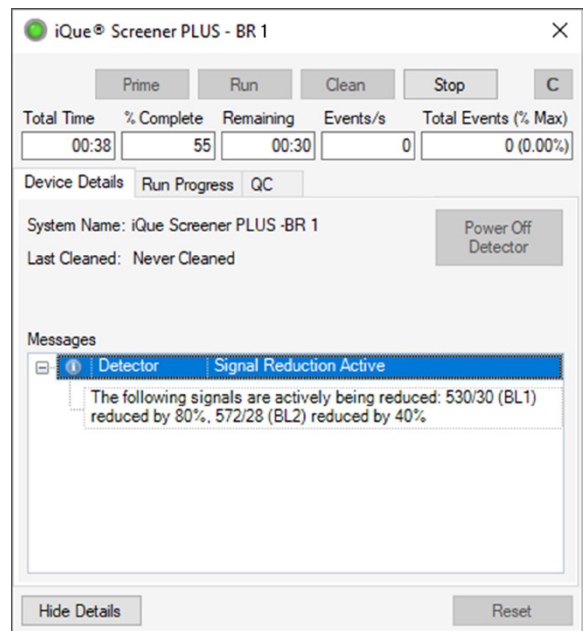
When an experiment is running which has Signal Reduction applied an informational message will be displayed to the user indicating which signals are actively being reduced. When the message is expanded details will appear indicating which signals are being reduced and by how much.

After the experiment finishes running the Signal Reduction will no longer be active and the Signal Reduction messages that were present in the Device Details tab will go away.

Each time a protocol is run the Signal Reduction will be specific to that protocol. Signal Reduction settings will be automatically cleared after each run.



The screenshot shows the iQue Screener PLUS - BR 1 interface. At the top, there are buttons for Prime, Run, Clean, and Stop. Below these are fields for Total Time (01:26), % Complete (46), Remaining (01:35), Events/s (0), and Total Events (% Max) (0 (0.00%)). The Device Details tab is selected, showing System Name: iQue Screener PLUS -BR 1 and Last Cleaned: Never Cleaned. A Power Off Detector button is visible. In the Messages section, a message titled 'Detector' with the sub-header 'Signal Reduction Active' is displayed.



The screenshot shows the iQue Screener PLUS - BR 1 interface with the 'Signal Reduction Active' message expanded. The expanded message text reads: 'The following signals are actively being reduced: 530/30 (BL1) reduced by 80%, 572/28 (BL2) reduced by 40%'. The interface elements are the same as in the previous screenshot, but the message is highlighted in blue.

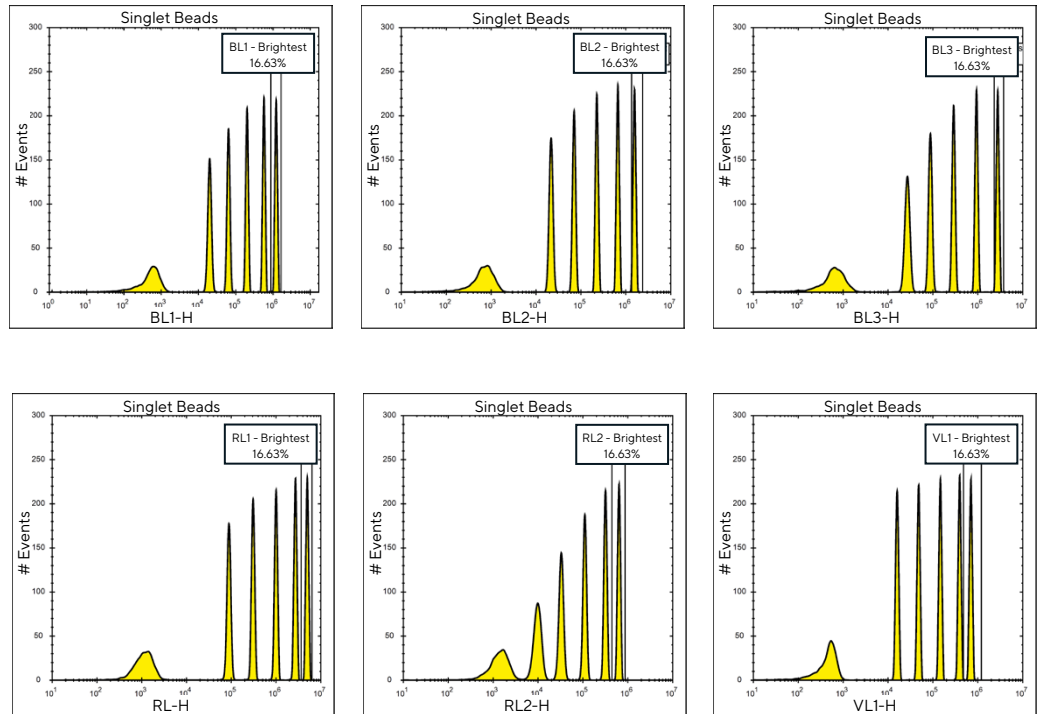
Example Data

The following table shows data for the VBR system and Signal Reduction settings for all channels at 0% and then again for all channels at 100%.

Instrument Details

Name: Lab iQue® 3
 Type: VBR
 Sample: PLUS QC Beads Tube
 Protocol: 5s@29RPM
 Signal Reductions
 - 445/45 (VL1): 0%
 - 530/30 (VL2/BL1): 0%
 - 572/28 (VL3/BL2): 0%
 - 530/30 (VL4/BL3): 0%
 - 530/30 (VL5/BL4/RL1): 0%
 - 530/30 (VL6/BL5/RL2): 0%

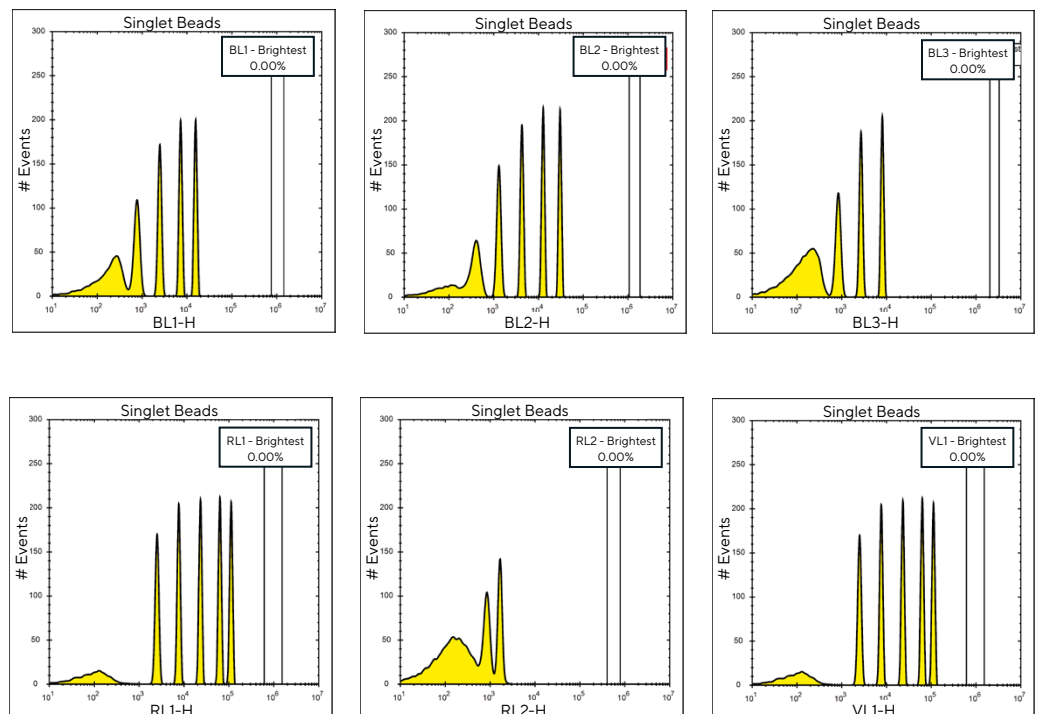
Histograms with gates at baseline (0% reduction)



Instrument Details

Type: VBR
 Sample: PLUS QC Beads Tube
 Protocol: 5s@29RPM
 Signal Reductions
 - 445/45 (VL1): 100%
 - 530/30 (VL2/BL1): 100%
 - 572/28 (VL3/BL2): 100%
 - 530/30 (VL4/BL3): 100%
 - 530/30 (VL5/BL4/RL1): 100%
 - 530/30 (VL6/BL5/RL2): 100%

Histograms with gates at baseline (100% reduction)



Experiment Documentation

When the Signal Reduction feature has been enabled plate summaries and Plate Reports will show the protocol information including the level to which the signal was reduced for each channel.

Plate Summary

The protocol and FCS header sections of the plate summary will include protocol information about Signal Reduction.

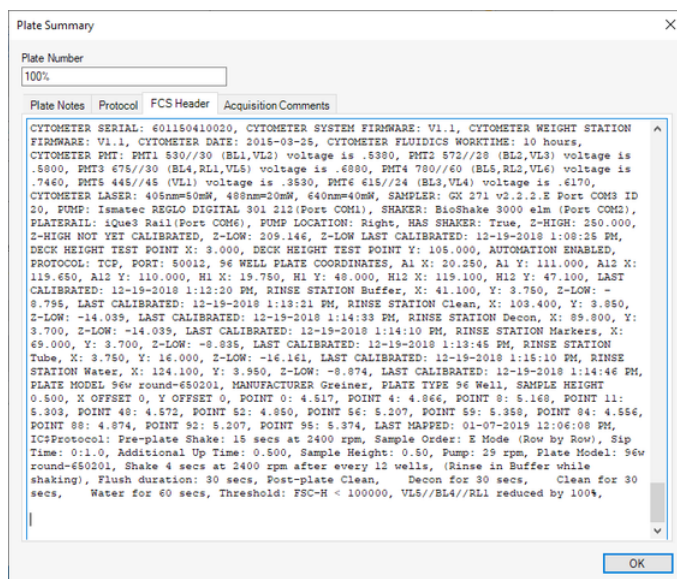
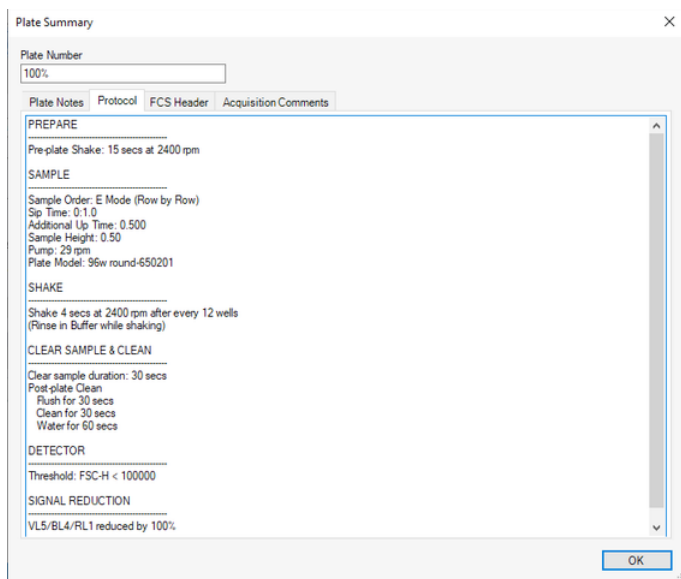


Plate Reports

The plate report Protocol section for Detector settings will also include information about any Signal Reduction that is specified on the plate.

Protocol

PREPARE

Pre-plate Shake: 15 secs at 2400 rpm

SAMPLE

Sample Order: E Mode (Row by Row)
Sip Time: 0:1.0
Additional Up Time: 0.500
Sample Height: 0.50
Pump: 29 rpm
Plate Model: 96w round-650201

SHAKE

Shake 4 secs at 2400 rpm after every 12 wells
(Rinse in Buffer while shaking)

CLEAR SAMPLE & CLEAN

Clear sample duration: 30 secs
Post-plate Clean
Flush for 30 secs
Clean for 30 secs
Water for 60 secs

DETECTOR

Threshold: FSC-H < 100000

SIGNAL REDUCTION

VL5/BL4/RL1 reduced by 100%

Sharing Signal Reduction Data

Experiments using the Signal Reduction feature can be exported as IEX or IET to other instances of Forecyt and analyzed even if the Signal Reduction feature is not licensed. However, non-licensed instruments will not be able to run and acquire data using Signal Reduction protocol settings for those experiments.

If non-licensed instruments attempt to run an experiment with Signal Reduction an error will occur requiring the activation of a valid license.

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