NAB-Sure™ SARS-CoV-2 Neutralizing Antibody Test Kit

Research Use Only

Instructions for Use

Data Analysis (for Bio-Rad PCR system)

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Disclaimer

This document and its contents are proprietary to Spear Bio and are intended solely for the use of customers in connection with the use of the product(s) described in the Terms of Use.

The instructions in this document must be strictly followed by qualified and properly trained personnel. All the contents of this document must be fully read and understood prior to using such product(s).

NAB-Sure™ SARS CoV-2 Neutralizing Antibody Test Kit is for research use only and is not intended for diagnostics purposes.

1. Introduction

NAB-Sure™ SARS-CoV-2 Neutralizing Antibody Test Kit is a neutralizing antibody (NAb) test for SARS-CoV-2 humoral immune response. The assay supports dried blood spot (DBS), plasma, and serum samples, which are analyzed using real-time PCR systems to produce a qualitative or quantitative (titer) result. NAB-Sure™ SARS CoV-2 Kit is a surrogate virus neutralization test.

2. About this Document

This document describes the steps to generate and analyze NAB-Sure™ data. The document has two parts. First, it describes the steps to set up the Bio-Rad qPCR platform to run the NAB-Sure™ assay. Second, it describes the steps and tools to analyze the output data and calculate the neutralization titer in whole blood eluates from dried blood spot (DBS) samples. The analysis requires the supplied Excel macro sheets, NABSure_macro_analysis_96wellplate.xlsx for 96-well plates and NABSure_macro_analysis_384wellplate.xlsx for 384-well plates.

3. Required Software

Table 1: Required Software

Software	Recommended	Minimum
Windows OS, Apple OS	Windows 10+, Apple OS X 10.10+	Windows 7, Apple OS X 10.10
Bio-Rad CFX Maestro™	CFX Maestro™ v2.3	CFX Maestro™ v2.3
Microsoft Excel Version	Excel 2021	Excel 2021

4. Data Analysis Workflow

Figure 1: Overview of the Data Analysis Workflow

5. Setting Up the qPCR plate

Before starting the Bio-Rad plate set up, please download the Spear Bio-created protocol and plate layout files, available at www.spear.bio/nabsurestart (*NABSure_PCR_protocol_BioRad.prcl, NABSure_96well_plate_BioRad.pltd*, and *NABsure_384well_plate_Biorad.pltd*). Follow the instructions on the website to create an account if one is not already established. When downloading the file, note where the file is saved on your computer. The steps below describe how to set up the NAB-Sure™ qPCR plate using the Bio-Rad CFX Real-Time PCR System and associated CFX Maestro™ software.

1. Open the CFX Maestro™ Software

2. Go to "File/Open/Protocol" to open the NAB-Sure™ protocol: *NABSure_PCR_protocol_BioRad.prcl*. Protocol window will be displayed as shown in Figure 2.

3. Confirm the qPCR protocol has the steps as described in Table 2.

Table 2: NAB Sure™ qPCR protocol steps for Bio-Rad instrument

Set up	Pre-heat lid @105 °C, forever (skip once the sample temperature reaches 95 °C)
Step 1	95 °C, 3 min
Step 2	95 °C, 15 sec
Step 3	55.9 °C, 1 min
Cycle steps 2 and 3	40 cycles
Step 4	10°C, 30 min (or more)

4. Once the protocol steps have been verified, select "OK"

5. In the "Run Set Up" window, select the "Plate" tab, and then click on "Select Existing" to open the plate layout template for 96 well-plate or 384-well plate (NABSure_96well_plate_BioRad.pltd or NABsure_384well_plate_Biorad.pltd, respectively).

6. Once the plate template is opened, click on "Edit Selected" to review the plate layout and verify FAM is selected as fluorophore. Then, select "OK". Figure 3 displays the NAB Sure™ 96-well plate layout.

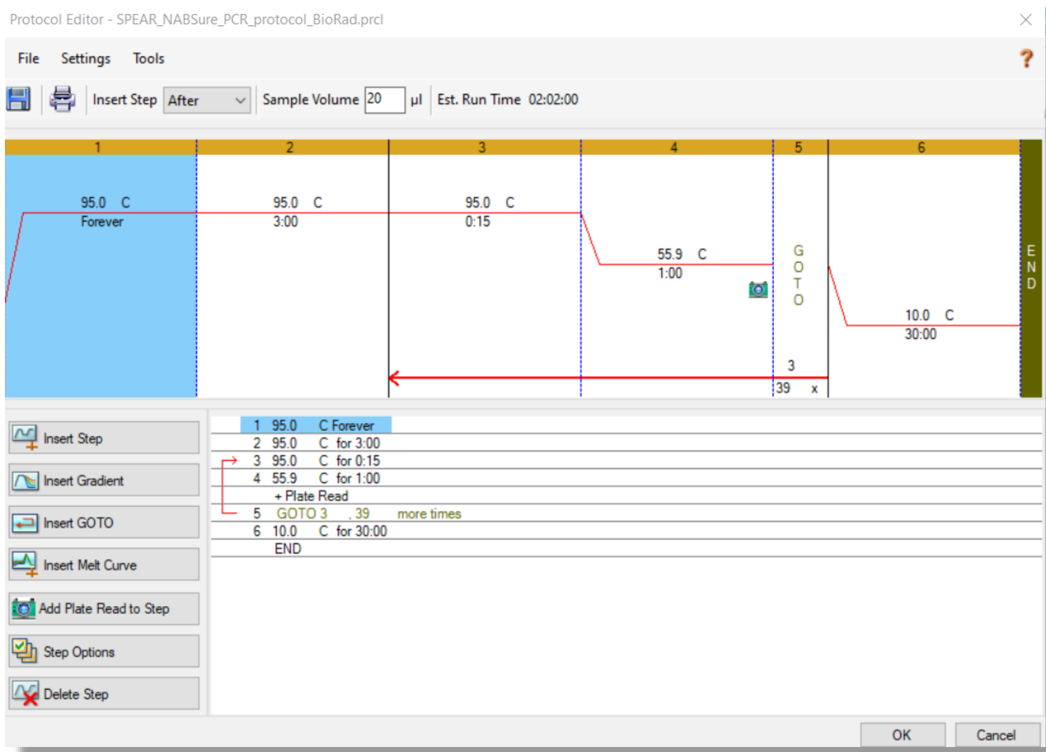

Figure 2: Protocol Editor Window of Bio-Rad CFX Maestro™

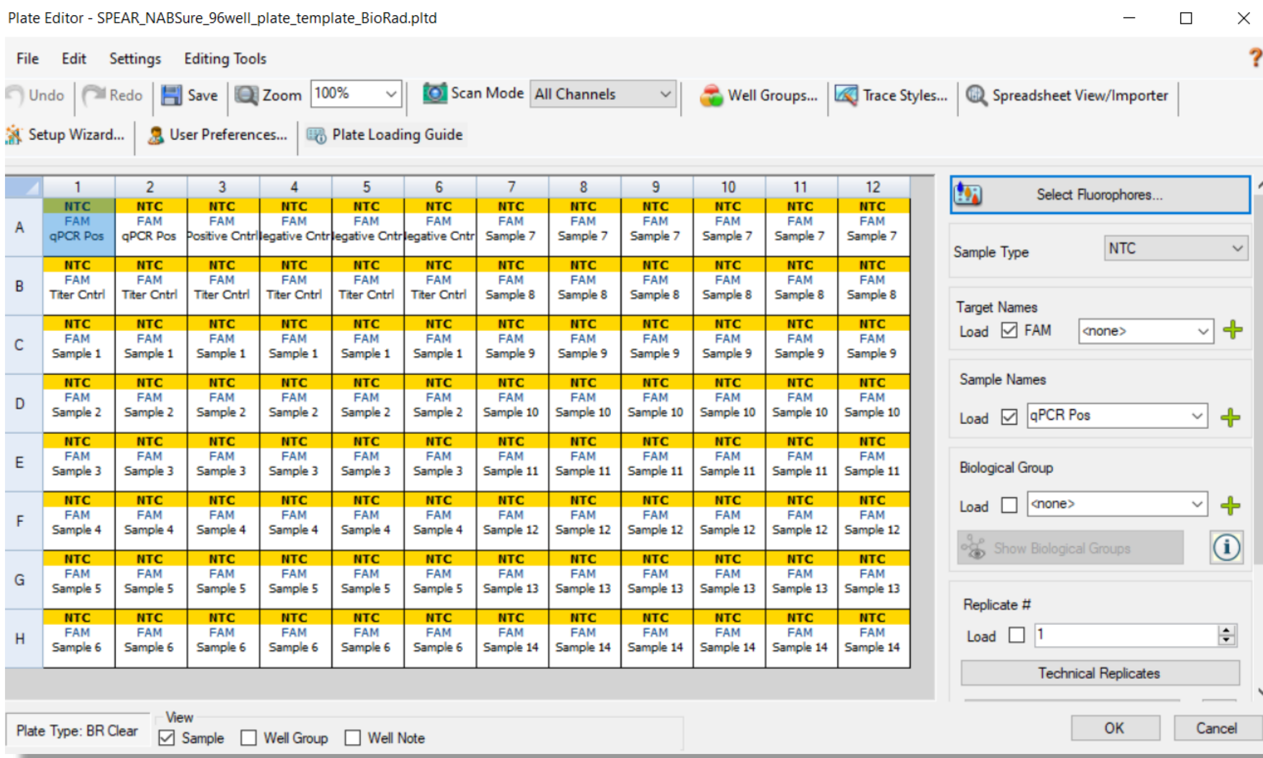

Figure 3: NAB Sure 96-well plate layout as displayed in Bio-Rad CFX Maestro™

7. In the “Run Setup” window, select the “Start Run” tab, select qPCR instrument and then, select the “Start Run” button at the bottom right corner, as shown in Figure 4.

8. User will be prompted to save the data in the desired location, then select “Save”

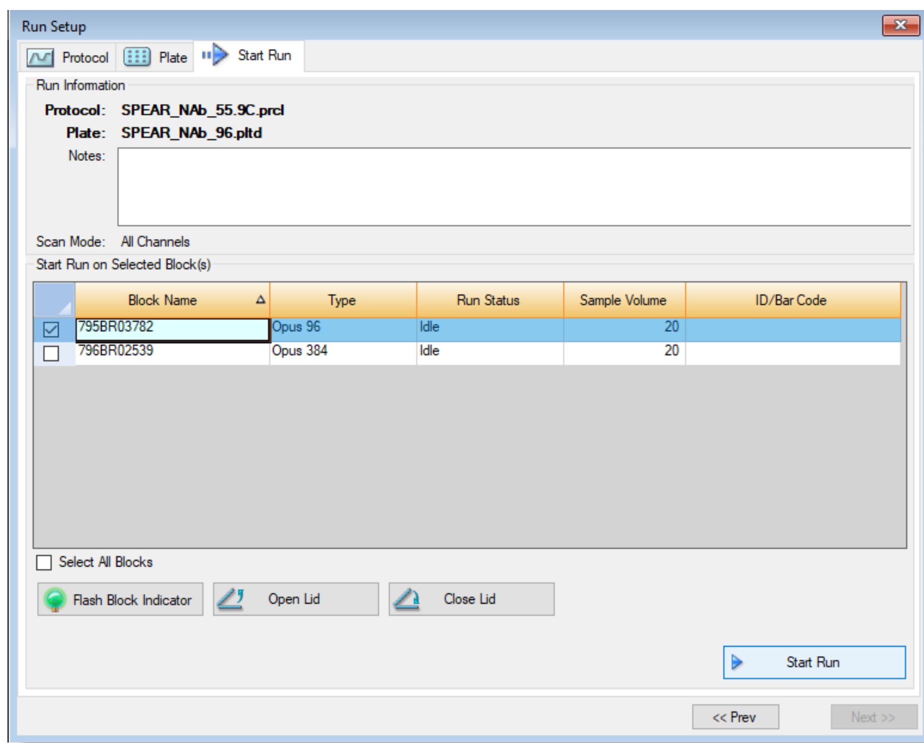

Figure 4: Start Run tab of Bio-Rad CFX Maestro™

9. The instrument will start preparing for the run. At this point, the lid and sample compartments will reach the programmed temperature.

10. Once the “Sample temperature” reaches 95°C, select “Open lid”

11. Insert qPCR plate inside the instrument

12. Select “Close lid”

13. Select “Skip step” to start step 1 of the qPCR program (see step 5.3 above).

6. Data Analysis Procedure

6.1. Export Data from the qPCR Instrument

1. Once the qPCR run has completed, stop the run, and then open the data file in the Bio-Rad CFX Maestro™ software.

2. Select "Log scale" to visualize qPCR cycle numbers in the equipment software.

3. Select qPCR positive control wells A1 and A2 and adjust the cycle number to 23 (average of both wells) by setting the threshold. This step is used to normalize cycle numbers across different runs (see Figure 5).

4. Export data into Excel file format by selecting the "Export" tab, and then "Export All Data Sheets", and "Excel 2007 (*.xlsx) as shown in Figure 5. Save the data in the desired location, and then transfer the file to the device that will run the analysis template.

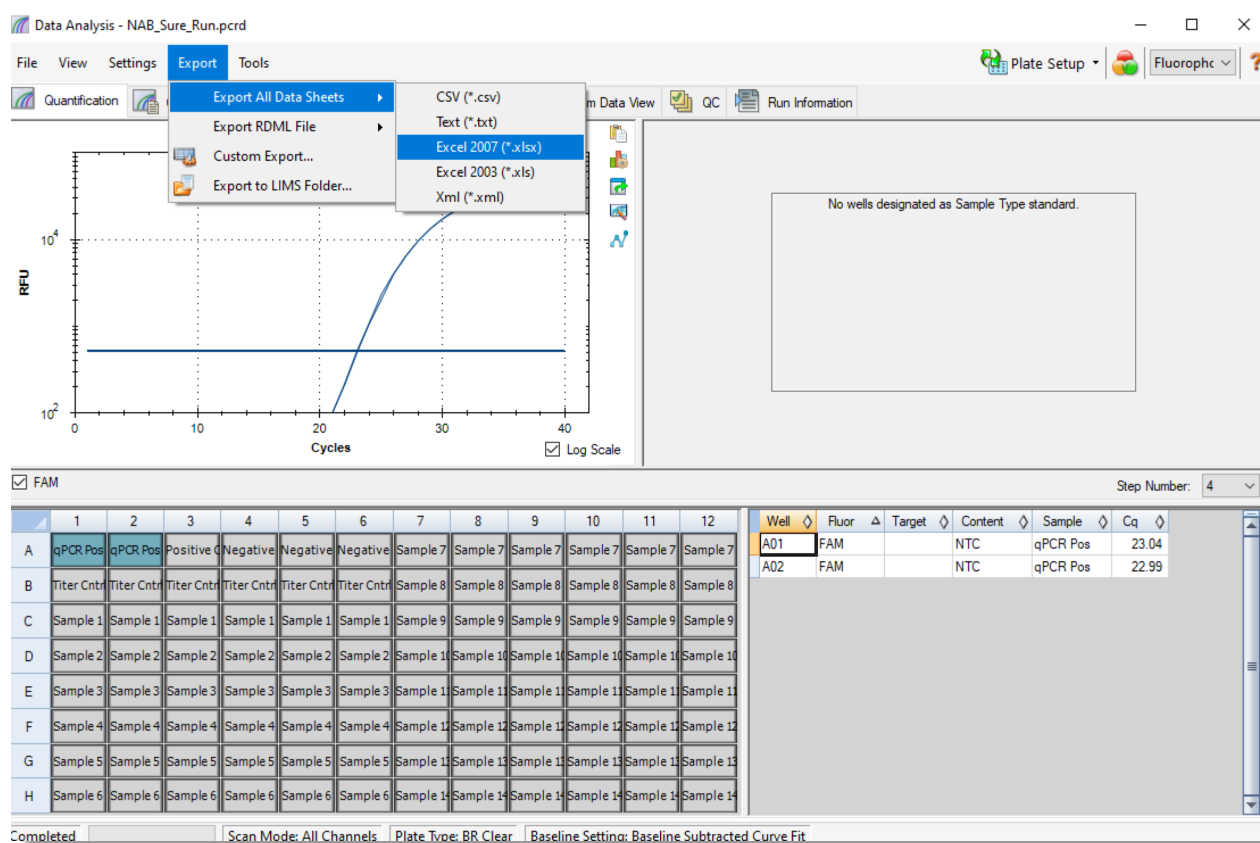

Figure 5: Normalization and exporting steps for NAB-Sure data using Bio-Rad qPCR

6.2. Open Spear Bio Analysis Template

Spear Bio provides an analysis template, available to download from the NAB Sure™ product support site (www.spear.bio/nabsurestart).

- Spear Bio's template files require Microsoft Excel's "Solver" function to be enabled. To enable this function, start the Excel program and access the View > Add Ins menu, then check the box "Analysis Toolpack".
- Once the "Solver" function has been enabled, open the "NABSure_macro_analysis_96wellplate.xlsx" for 96-well plate or the "NABSure_macro_analysis_384wellplate.xlsx" for 384-well plate.

The Analysis Template has two tabs: "Dashboard" and "Sample Analysis".

The "Dashboard" tab contains the main controls and displays the overall results once the analysis completes. The "Dashboard" tab has the following sections:

- Load New Data: Reads and processes qPCR data in the current sheet.
- QC Summary: Displays the quality control metrics.
- Analysis Options: Manages processing of negative controls and dilution factor options.
- Recalculate: Fits inhibition curves to data and finds the dilution with 50% inhibition (NT50).
- Export: Saves the processed data in a fresh spreadsheet without macros.
- Sample by Sample Results. Displays the "Sample Table" with NT50 values for each sample and any QC notes.

The "Sample Analysis" tab displays the "Sample Table", and the titer curves (including inhibition percentages per sample and dilution).

6.3. Load Data

1. Select "Load New Data" and navigate to the appropriate Bio-Rad data file ("Quantification Cq Results" or "Quantification Plate View Results"), then select "Open" (see Figure 6).

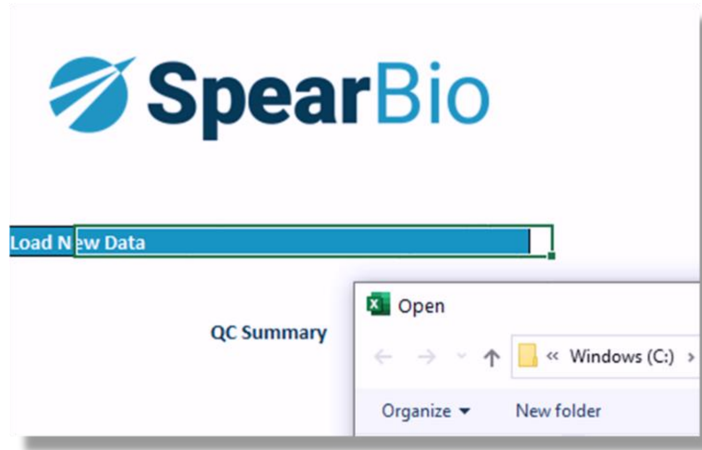

Figure 6: Spear Bio Template Load File

2. A pop-up window will be displayed to confirm that the specified data file has been loaded, then select “OK”.

Data are read from an external spreadsheet exported from the Bio-Rad instrument. In all cases, data are expected to conform to a standard plate layout (Figures 7 and 8), with control samples at upper left and samples in groups of six laid out on rows of the plate. Data for all wells are expected to be present in the xlsx file, even if a partial plate is processed, with 0 or N/A as the Ct value for the unused wells.

The plate layout is shown below:

Legend

Q+	qPCR positive control
S+	Positive control sample
S-	Negative control sample
TC	Titer control sample
S1-S62	Experimental samples

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12
Row A	Q+	Q+	S+	S-	S-	S-	S7	S7	S7	S7	S7	S7
Row B	TC	TC	TC	TC	TC	TC	S8	S8	S8	S8	S8	S8
Row C	S1	S1	S1	S1	S1	S1	S9	S9	S9	S9	S9	S9
Row D	S2	S2	S2	S2	S2	S2	S10	S10	S10	S10	S10	S10
Row E	S3	S3	S3	S3	S3	S3	S11	S11	S11	S11	S11	S11
Row F	S4	S4	S4	S4	S4	S4	S12	S12	S12	S12	S12	S12
Row G	S5	S5	S5	S5	S5	S5	S13	S13	S13	S13	S13	S13
Row H	S6	S6	S6	S6	S6	S6	S14	S14	S14	S14	S14	S14

Figure 7: Plate data format for 96-well plate

	Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12	Col 13	Col 14	Col 15	Col 16	Col 17	Col 18	Col 19	Col 20	Col 21	Col 22	Col 23	Col 24
Row A	Q+	Q+	S+	S-	S-	S-	S15	S15	S15	S15	S15	S15	S31	S31	S31	S31	S31	S31	S47	S47	S47	S47	S47	S47
Row B	TC	TC	TC	TC	TC	TC	S16	S16	S16	S16	S16	S16	S32	S32	S32	S32	S32	S32	S48	S48	S48	S48	S48	S48
Row C	S1	S1	S1	S1	S1	S1	S17	S17	S17	S17	S17	S17	S33	S33	S33	S33	S33	S33	S49	S49	S49	S49	S49	S49
Row D	S2	S2	S2	S2	S2	S2	S18	S18	S18	S18	S18	S18	S34	S34	S34	S34	S34	S34	S50	S50	S50	S50	S50	S50
Row E	S3	S3	S3	S3	S3	S3	S19	S19	S19	S19	S19	S19	S35	S35	S35	S35	S35	S35	S51	S51	S51	S51	S51	S51
Row F	S4	S4	S4	S4	S4	S4	S20	S20	S20	S20	S20	S20	S36	S36	S36	S36	S36	S36	S52	S52	S52	S52	S52	S52
Row G	S5	S5	S5	S5	S5	S5	S21	S21	S21	S21	S21	S21	S37	S37	S37	S37	S37	S37	S53	S53	S53	S53	S53	S53
Row H	S6	S6	S6	S6	S6	S6	S22	S22	S22	S22	S22	S22	S38	S38	S38	S38	S38	S38	S54	S54	S54	S54	S54	S54
Row I	S7	S7	S7	S7	S7	S7	S23	S23	S23	S23	S23	S23	S39	S39	S39	S39	S39	S39	S55	S55	S55	S55	S55	S55
Row J	S8	S8	S8	S8	S8	S8	S24	S24	S24	S24	S24	S24	S40	S40	S40	S40	S40	S40	S56	S56	S56	S56	S56	S56
Row K	S9	S9	S9	S9	S9	S9	S25	S25	S25	S25	S25	S25	S41	S41	S41	S41	S41	S41	S57	S57	S57	S57	S57	S57
Row L	S10	S10	S10	S10	S10	S10	S26	S26	S26	S26	S26	S26	S42	S42	S42	S42	S42	S42	S58	S58	S58	S58	S58	S58
Row M	S11	S11	S11	S11	S11	S11	S27	S27	S27	S27	S27	S27	S43	S43	S43	S43	S43	S43	S59	S59	S59	S59	S59	S59
Row N	S12	S12	S12	S12	S12	S12	S28	S28	S28	S28	S28	S28	S44	S44	S44	S44	S44	S44	S60	S60	S60	S60	S60	S60
Row O	S13	S13	S13	S13	S13	S13	S29	S29	S29	S29	S29	S29	S45	S45	S45	S45	S45	S45	S61	S61	S61	S61	S61	S61
Row P	S14	S14	S14	S14	S14	S14	S30	S30	S30	S30	S30	S30	S46	S46	S46	S46	S46	S46	S62	S62	S62	S62	S62	S62

Figure 8: Plate data format for 384-well plate

Data can be imported from two different formats:

- Generic format - The workbook has a sheet with two columns, one labeled “Well” and the other “Ct/Cq” in the first row.   If there are multiple columns containing Ct or Cq, (e.g. Ct mean, Ct std. dev.), the first column will be used as the cycle count.
- Bio-Rad format - Data are exported from the “Quantification Plate View Results” instrument in a format where there are four rows per row of the plate and one column per column of the plate.   The Cq is in the third row for each sample.   The well location is inferred from the location of the cell in the sheet exported from the instrument. Data can also be exported from the “Quantification Cq Results” file, which follows the Generic format explained above.

6.4. QC Summary

Once the data file has been loaded, the analysis template generates quality control metrics for the specified dataset. The QC Summary includes three sections: Positive Controls, Negative Controls and Experimental Samples. There are two types of warning labels depending on the type of QC metric. Warnings with a red label imply that the assay likely failed whereas warnings with an amber label imply that performance of the assay has not been optimal, but results can still be reported.

Positive Controls

- qPCR Positives: The mean and difference of the Ct values from the two qPCR positive controls are displayed in this section. The difference between the mean of the qPCR positive control and the mean of the negative sample control should be between 1 and 4. If the difference is out of this range, a warning will be displayed in amber color.

  The difference between the Ct values from the two qPCR positive controls should be less than 0.3. If that is not the case, the “Diff” value will be flagged in amber. For more information, please refer to the *Troubleshooting* document (available at www.spear.bio/nabsurestart).

- Positive Control Sample: The positive control sample is expected to be at least 7 cycles more than the mean UN1 of the negative control samples. If that is not the case, the "PosC-NegC" value will be flagged in red. For more information, please refer to the *Troubleshooting* document (available at www.spear.bio/nabsurestart).

Negative Controls

- The Mean UN1 is the mean of the negative control samples. The negative control samples should have a standard deviation less than 0.2. If that is not the case, the SD value will be flagged in amber.

- The Mean Inferred Negative is the mean of the lower asymptotes of the sample Ct curves. Individual negatives are calculated from samples that saturate. The average of such values is the inferred negative.
- The overall mean (UN2) is the mean of the negative control samples and any inferred negatives from curves that have reached their inhibition limit, as described below in the Analysis Options section.

Experimental Samples

This section displays the number of samples that fail QC metrics (neutralization titers were suspect or undetermined). For more information, refer to the Data Interpretation section.

QC Summary

Positive Controls			
qPCR Positives		Positive Control Sample	
Mean	Diff	Mean	PosC-NegC
23.06	0.04	29.06	7.34
Negative Controls			
Mean (UN1)	SD	Mean Inferred Neg	Overall mean (UN2)
21.72	0.03	21.72	21.72
		Asymptotes: 5	
Experimental Samples			
Failed QC	0		

Figure 9: The QC Metrics Table per Plate Analyzed

6.5. Analysis Options

The "Analysis Options" section contains default parameters based on the assay and workflow characteristics (specified in the *NAB-Sure SARS-CoV-2 Assay - Instructions for Use for 96- or 384-well plate*). There are four options that the user can modify in this section:

- Sample Dilution: The dilution factor from the sample. The default value is 50, which corresponds to the estimated dilution factor from a dried blood sample. For serum samples, this number is usually between 10 - 100. The value can be changed in the appropriate cell.
- Starting Serial Dilution: The dilution series for each sample is set to start at 1x. This can be changed in the appropriate cell.
- Serial Dilution Step: Default is 3x stepping for each subsequent dilution. This can be changed in the appropriate cell.
- Negative Strategy (Background subtraction). There are three options for background subtraction:
    - Universal negative #1 (UN1): The mean of the Ct values of the three negative control samples. This method is the default method for calculating the negative reference.
    - Universal negative #2 (UN2): The mean of the Ct values of the three negative control samples and the Ct values of inferred negative samples that are determined by a proprietary algorithm. This method is recommended if:
        - A broadly-based negative reference using more data points is desired. This is particularly useful if the standard deviation of the three negative control samples is high (e.g., > 0.15) or the values contain outliers or the values are skewed (e.g., visualize the curves and the lower asymptote are all above 10% or lower than -10%).
        - The samples have sample matrix effects, and the Ct values of negative control samples deviate from the actual sample negatives (e.g., visualize the curves and the lower asymptote are all above 10% or lower than -10%).
        - Be aware that such a negative theoretically introduces inter-dependency among samples, in that the data for one sample can affect the negative used for all samples. This inter-dependency is likely a second-order consideration, since the negative value from each sample is only used if it is not too far from the negative control samples.
    - Individual negative (IN): The negative value is determined for each individual sample by a proprietary algorithm. This method is recommended if individual sample curves show parallel shifting up or down, which can be occasionally

observed when using manual protocol. The parallel shifting can be determined by visualizing the curves and checking the lower asymptote (more than 10% deviating from 0% inhibition).

To change the background subtraction, select the UN1 default cell, and then click on the dropdown icon to display the other two options for background subtraction.

Figure 10: Dilution Factor and Background Correction Options

6.6. Recalculate

- Once the analysis options have been selected, click on “Recalculate” to analyze the imported data.
- A pop-up window will be displayed with the message *“Recalculating curve fits. This might take a few seconds”*. Users need to select “OK” to run the analysis.
- The analysis may take 10 to 60 seconds depending on the computing power of the analysis machine.
- Once the analysis completes, results will be displayed in the “Sample Table” at the bottom of the “Dashboard” tab, see Figure 11.
- In the “Sample Table”, the NT50 value for each sample and any QC notes are listed. A quality control column (“QC PASS”) flags any unusual features for that sample. If a sample metric fails QC, we recommend removing the affected samples. Please refer to Table 3 for data interpretation.

Sample by sample results

Sample Table				
Name	NT50	NT50 (blood)	QC pass	Comments
Titer Control	7.42	370.96	TRUE	
S1	8.99	449.64	TRUE	
S2	7.60	380.21	TRUE	
S3	13.71	685.38	TRUE	
S4	13.51	675.59	TRUE	
S5	9.73	486.45	TRUE	
S6	7.46	373.19	TRUE	
S7	11.61	580.34	TRUE	
S8	9.03	451.63	TRUE	
S9	-	-	FALSE	Undetectable, R2 < 98%
S10	-	-	FALSE	Undetectable, R2 < 98%
S11	3.11	155.34	TRUE	
S12	2.64	131.77	TRUE	
S13	8.87	443.55	TRUE	
S14	8.82	441.00	TRUE	

Figure 11: Sample Table with Example Data Output

6.7. Export Results

The “Export Results” button allows the processed data to be saved to a fresh spreadsheet without macros, formulas, or references to other spreadsheets. Pressing the export button prompts for a filename before saving the data.

6.8. Sample Analysis Tab

Data are also visualized on a second tab, “Sample Analysis”. This tab contains the “Sample Table”, the chart displaying the curves and the inhibition data for each sample.

Next to the “Sample Table”, there are two columns associated with the chart display. There is a checkbox (Y/N) to request a titer curve for a given sample to be added to the chart (see Figure 12). By default, all 15 curves (including the titer control) are displayed in the chart of the 96-well analysis template. In the case of the 384-well analysis template, the first 15 samples (including the titer control) are displayed. There is a drop-down menu to select other chart displaying options (described below).

- Check All Samples, only available in the 96-well analysis template. It displays all 15 curves, including the titer control. This is the default option for the 96-well template.
- Uncheck All Samples: It deselects all samples
- Check Failed QC samples: It only displays the curves from the samples that did not pass the QC metrics.

- Group 1 (TC-S15), only available in the 384-well analysis template. It displays the curves from the first 15 samples, including the titer control (from Column 1 of 384-well plate). This is the default option for the 384-well analysis template.
- Group 2 (S16-S31), only available in the 384-well analysis template. It displays the curves from the second set of samples (from Column 7 of 384-well plate).
- Group 3 (S32-S47), only available in the 384-well analysis template. It displays the curves from the third set of samples (from Column 13 of 384-well plate).
- Group 4 (S48-S62), only available in the 384-well analysis template. It displays the fourth set of samples (from Column 19 of 384-well plate).

Sample Table						
Name	NT50	NT50 (blood)	QC pass	Comments	UPDATE GRAPH	Check all samples
Titer Control	6.25	312.34	TRUE		Y	
S1	6.15	307.40	TRUE		Y	
S2	1.00	50.00	FALSE	Ultralow Titer	Y	
S3	36.65	1,832.69	TRUE		Y	
S4	89.88	4,494.10	TRUE		Y	
S5	243.00	12,150.00	FALSE	Ultrahigh Titer	Y	
S6	243.00	12,150.00	FALSE	Ultrahigh Titer	Y	
S7	26.17	1,308.66	FALSE	Sudden drop, R2 < 98%	Y	
S8	243.00	12,150.00	FALSE	Ultrahigh Titer	Y	
S9	243.00	12,150.00	FALSE	Ultrahigh Titer	Y	
S10	11.16	558.14	FALSE	R2 < 98%	Y	
S11	243.00	12,150.00	FALSE	Ultrahigh Titer	Y	
S12	243.00	12,150.00	FALSE	Ultrahigh Titer	Y	
S13	243.00	12,150.00	FALSE	Ultrahigh Titer, Flat slope, R2 < 98	Y	
S14	243.00	12,150.00	FALSE	Ultrahigh Titer, Sudden drop	Y	

Figure 12: Sample Table on Sample Analysis Tab

In the chart, inhibition data for the selected samples are shown with square markers. The fitted curves for the samples are shown with lines of matching color (see Figure 13).

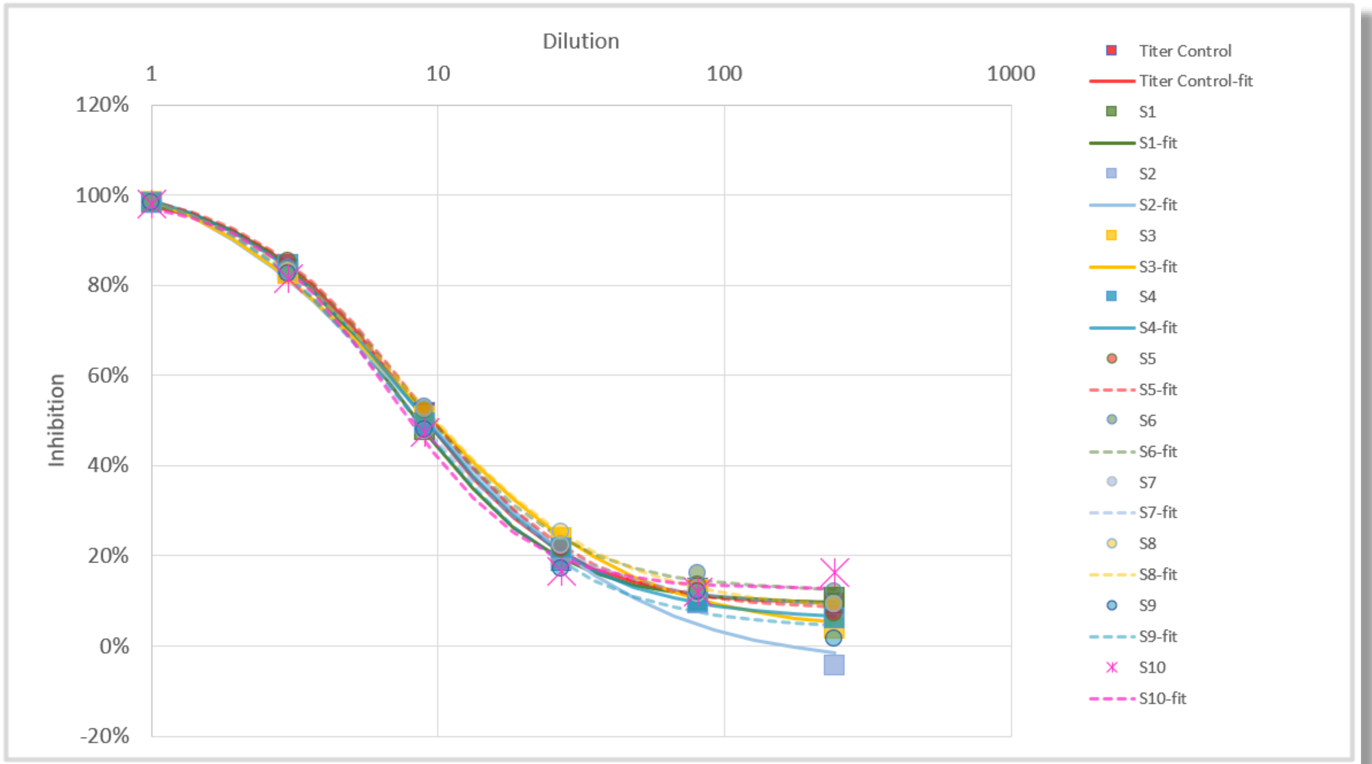

Figure 13: Example of Chart on Sample Analysis Tab

7. Data Interpretation

Table 3 lists critical data interpretation for titer results and quality control metrics.

Table 3: Data Interpretation for Titer Results and Quality Control Metrics

Metric	Description
Large variation between the two qPCR Positive Control replicates (Difference > 0.3)	This could be due to inaccurate pipetting or instrument not calibrated. For more information, refer to the *Troubleshooting* document.
Is the Positive Control Sample – Negative Control Sample difference larger than 7.0?	If not, the quality of the run may be inadequate. For more information, refer to the *Troubleshooting* document.

Large standard deviation of Negative Control Sample Ct values (SD > 0.2)	• Is there any outlier in the three data points that can be removed from calculation? • Use overall mean (UN2) instead of mean UN1 as the reference for calculating inhibition percentages.
Difference between Mean qPCR positive control and Mean of Negative Control Samples UN1 is outside the range 1-4	• This could be due to inaccurate pipetting or instrument not calibrated • Is there any outlier in the three data points from the negative controls that can be removed from calculation? • Is this the only QC metric that failed?
Titer value of the Titer Control Sample is out of the range 8 ± 20%.	Issues with dilution step or negative reference value is off. Check the curve of the Titer Control Sample to see if the asymptote is more than 10% off 0% inhibition. Recalculate the titer using the UN2 or IN background subtraction method.
Ultrahigh Titer	All inhibition results are over 50%. A placeholder of the highest dilution (243) is used for interpolated NT50.
Ultralow Titer	Highest inhibition results > 40% but < 50%. A placeholder of the lowest dilution (1) is used for interpolated NT50.
Undetectable	Highest inhibition is < 40%.
Sudden drop on a dilution curve	A sudden drop on a curve means that the measured inhibition went from high inhibition to low inhibition in just one or two dilutions. This should be considered a potential user error.
R2 < 98%	The curve fit is not optimal, most likely due to outliers. The NT50 value may not be accurate.
Flat slope	The results for serial dilutions are unusually similar to each other, that is, dilution has little effect. This should be considered a potential sample problem.

8. Frequently Asked Questions

Q. What is the NT50 value?

A. The assay is a signal-off assay. An increase in the neutralizing capability of a sample causes reduced interaction between the two SPEAR probes (S1 and ACE2), resulting in a decrease in SPEAR signals. Using a negative sample containing no neutralizing antibodies as a reference, the inhibition is calculated by the signal reduction compared to the reference. The NT50 value is the dilution factor at which the inhibition of the diluted sample reaches 50%. To calculate NT50, the inhibition values of a serial diluted samples are fitted to a 4PL curve and the point of 50% inhibition is estimated by interpolation from this curve.

Q. How does the template convert Ct values to inhibition percentages?

A. The cycle number difference between the negative reference and the measured dilution point is calculated. The cycle number difference is converted to "Inhibition" using the following equation:

$$Inhibition = \left(1 - \frac{1}{2^{Cycle\ number\ difference}}\right) * 100\%$$

Q. How does the template calculate NT50 Whole Blood values?

A. DBS samples are eluted using DBS elution buffer and therefore diluted from the original whole blood. The NT50 whole blood values are calculated to represent the neutralization titer in the whole blood. We estimate the dilution factor from the original blood to the DBS eluate is ~50 for a ¼" DBS punch when eluted using 500 μL of buffer. Therefore,

$$NT50\ in\ whole\ blood = Interpolated\ NT50\ \times 50$$

Q. How does the template fit an inhibition curve?

A. Curve Fit Methodology
Once data are loaded, the solver is invoked to process the data for the plate. The raw data is background subtracted and converted to inhibition values.

The inhibition curves are fit with a 4 parameter (4PL) curve according to the formula

$$Y = B + \frac{T - B}{1 + \left(\frac{x}{X_{50}}\right)^m}$$

The value B is the lower asymptote of the inhibition curve (usually close to 0%), T is the upper asymptote (normally ~100%). The parameter m is the slope of the curve (usually between 1 and 2) and X50 is the dilution at which the curve is halfway between T and B (normally NT50 is close

to X50). The 50% neutralization (NT50) is extracted by finding the dilution where the inhibition curve intersects 50%.

Q. What results are reported if the inhibition curve fit is incomplete?

A. For high titer samples where no dilution comes down to 50% inhibition, a placeholder of 243 is used for interpolated NT50.   For low titer samples where, highest inhibition reaches 40% but not 50%, a placeholder of 1 is used for interpolated NT50. For samples where highest inhibition does not reach 40%, samples are marked as “Undetectable”.

Q. Can I set up the qPCR plate in a different configuration to the template in Figures 7 or 8?

A. Missing samples can be accommodated; however, all sample dilutions must be 6 points and descend from wells, e.g. Column 1 - Column 6, or Column 7 - Column 12. The qPCR positive controls must be placed in Well A1 and A2. The Positive Control Sample must be placed in Well A3. The Negative Control Samples must be placed in wells A4 – A6.

Q. What error modes are reported by the template?

A. Refer to the Data Interpretation section.

9. Support

For all technical support inquiries, please contact support@spear.bio or 781-937-5245

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