

Biomark Pathogen Detection Software

For use with the Advanta SARS-CoV-2 Mutation Assay Panel and the Advanta Preamp & IFC Reagent Kit—192.24 Kit

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About the Software

The Biomark Pathogen Detection Software is a command-line software tool used to interpret real-time PCR data generated by Biomark™ HD and the Real-Time PCR Analysis software when following the Advanta™ SARS-CoV-2 Mutation Assay Panel Protocol (FLDM-00479). The Biomark Pathogen Detection Software is for use only with Table Results exported from the Real-Time PCR Analysis software v4.7.1 or later. The Biomark Pathogen Detection Software can either be initiated within the Real-Time PCR Analysis software as a post export command or run separately from a command line.

The Detector Setup must be annotated in the Real-Time PCR Analysis software using the following names:

N1	N2	RNase P
E484K Wild Type	K417N Wild Type	Del69&70 Wild Type
E484K Mutant	K417N Mutant	Del69&70 Mutant
N501Y Wild Type	K417T Wild Type	L452R Wild Type
N501Y Mutant	K417T Mutant	L452R Mutant

The Biomark Pathogen Detection Software uses these assays to interpret results.

Computer Requirements


The minimum computer requirements for running the Biomark Pathogen Detection Software are:

- Operating system: Microsoft® Windows® 10 (32- or 64-bit)
- CPU: Intel® Core™ i5 or later
- Memory: 4 GB of RAM

Software and Input File Requirements

The Biomark Pathogen Detection Software v2.1.1 can only interpret files exported as **Table Results (*.csv)** from the Real-Time PCR Analysis software version v4.7.1 or later.

Install the Biomark Pathogen Detection Software

- 1 Download the software installer file from fluidigm.com/software.
- 2 Double-click the Biomark Pathogen Detection Software Setup icon .
- 3 Follow the wizard prompts to accept the license agreement and install the software.

The Biomark Pathogen Detection Software Setup application is for use only in installing or uninstalling the software. Double-clicking the icon does not run the software. You can delete the setup application after the software is installed.

You can either set up the Real-Time PCR Analysis software to export data directly through the Biomark Pathogen Detection Software or you can run the Biomark Pathogen Detection Software from a command line to interpret the Ct results and save the interpretation as a CSV file.

Use the Biomark Pathogen Detection Software



Before using the Biomark Pathogen Detection Software, annotate the real-time PCR data as described in the Advanta SARS-CoV-2 Mutation Assay Panel Protocol (FLDM-00479).

Option 1: Run the Biomark Pathogen Detection Software from the Real-Time PCR Analysis Software

To run the Biomark Pathogen Detection Software from the Real-Time PCR Analysis Software:

- 1 Set up the post export command in the Real-Time PCR Analysis software:
 - NOTE:** Setting up the post export command is only required before the first analysis unless settings for this option have been changed between analyses.
 - a** In the Real-Time PCR Analysis software, select **Tools > Options > Output > Results Export**.
 - b** (Optional) Specify a default folder for the exported results files.
 - c** Check the **Export Sample and Reagent Inlet to Plate Mapping** checkbox to report the plate quality control (QC) results in the output file.
 - NOTE:** See the Real-Time PCR Analysis User Guide (68000088) for information about the other checkboxes
 - d** Check the **Use Post Export Command** checkbox, then browse to:
C:\Program Files (x86)\Fluidigm\Biomark Pathogen Detection Software\
 - NOTE:** If you are running the 32-bit version of the Windows operating system, browse to:
C:\Program Files\Fluidigm\Biomark Pathogen Detection Software
 - e** Select the **BiomarkPD** application.
 - f** In the Additional Arguments field, enter **-panel RUO_N1_N2_Mutations -re**
 - NOTE:** Entering “-re” in the Additional Arguments field of the Real-Time PCR Analysis software enables the software to display a message when the Biomark Pathogen Detection encounters an error. Be sure to separate the 2 arguments with a space (-panel RUO_N1_N2_Mutations -re).
 - g** Confirm that the **Prompt for confirmation before execution** checkbox is checked, then click **OK**.
- 2 After annotating the real-time PCR data, export the analysis results as type **Table Results (*.csv)** to interpret the Ct results and save them to the designated CSV file.
- 3 Click **OK** when prompted to execute the post export command.

Option 2: Run the Biomark Pathogen Detection Software from a Command Line

Set up the export options in the Real-Time PCR Analysis software:

- 1 In the Real-Time PCR Analysis software, select **Tools > Options > Output > Results Export**.
- 2 (Optional) Specify a default folder for the exported results files.
- 3 Check the **Export Sample and Reagent Inlet to Plate Mapping** checkbox to report the plate quality control (QC) results in the output file.

To run the Biomark Pathogen Detection Software from a command line:

- 1 After annotating the real-time PCR data, export the analysis results as type **Table Results (*.csv)** to interpret the Ct results and save them to the designated CSV file.
- 2 In the Windows taskbar, select **Start button > Windows System > Command Prompt**.
- 3 In the Command Prompt window, change the directory to the C drive, if necessary.
- 4 Navigate to the folder that contains the Biomark Pathogen Detection Software:

```
cd "\Program Files (x86)\Fluidigm\Biomark Pathogen Detection Software"
```

NOTE: If you are running the 32-bit version of the Windows operating system, enter:
cd "\Program Files\Fluidigm\Biomark Pathogen Detection Software"

- 5 Run the command:

```
BiomarkPD [input filename] [output filename] -panel RU0_N1_N2_Mutations
```

NOTE: Do not include the brackets ([]) when entering the filenames; they are not part of the command.

This command interprets the input .csv file (exported from Real-Time PCR Analysis software) and saves it to the output .csv filename. Include the directory information when entering the filename.

NOTE: If the output filename is not specified, the input file will be overwritten with the interpretive results. If the input file is overwritten, open the Real-Time PCR Analysis software and export it again.

NOTE: To display the name, version, and build number for the Biomark Pathogen Detection Software, enter: **BiomarkPD -nvb**

Interpretation



For more information about the interpretation of results, see the Advanta SARS-CoV-2 Mutation Assay Panel Protocol (FLDM-00479).

Specifications

- Maximum positive Ct: A Ct of 32 is the maximum value allowed to call a reaction positive.
- Number of positives: Requires 2 or more positive replicate reactions to call an assay positive.
- Interpretive assays: Must be named **N1, N2, RNase P, E484K Wild Type, E484K Mutant, N501Y Wild Type, N501Y Mutant, K417N Wild Type, K417N Mutant, K417T Wild Type, K417T Mutant, Del69&70 Wild Type, Del69&70 Mutant, L452R Wild Type, and L452R Mutant.**

N1, N2, and all the Wild Type and Mutation assays must be type **Test**.
RNase P assay must be type **Control**.
- Empty assays: Must be named **Empty** and must be type **Blank**.
- Test samples: Must be of type **Unknown**.
- Control samples: Must be named **PC Mutant, PC Wild Type, NC, NTC.**
No template control must be of type **NTC**.
Negative and positive control must be of type **Unknown**.
- Empty samples: Must be of type **Blank**.
- Quality Threshold: Must be **0.65**.
- Baseline Correction: Must be set to **Linear***.
- Ct Threshold Method: Must be set to **Auto By Control (Global)***.

* You can set these as the default selections in the Real-Time Analysis PCR software. See the Advanta SARS-CoV-2 Mutation Assay Panel Protocol (FLDM-00479) for more information.

Interpretation of Control Results

NOTE: + = Detected, – = Not detected; Results must match table below to be considered passing

Control Description	Assay Result									Control Result
	N1	N2	RNase P	Del69&70	G22813T (K417N)	A22812C (K417T)	T22917G (L452R)	G23012A (E484K)	A23063T (N501Y)	
Positive control for wild type assays (PC Wild Type)	+	+	+	Absent	Absent	Absent	Absent	Absent	Absent	Pass
Positive control for mutant assays (PC Mutant)	+	+	+	Present	Present	Present	Present	Present	Present	Pass
Negative control (NC)	–	–	+	No Template	No Template	No Template	No Template	No Template	No Template	Pass
No template control (NTC)	–	–	–	No Template	No Template	No Template	No Template	No Template	No Template	Pass

Interpretation of Patient Specimen Results

A mutation assay pair is interpreted as detected (+) if either the wild type or mutation assay is detected (+). A mutation assay pair is interpreted as not detected (–) if either the wild type or mutation assay is not detected (–).

The plate QC results that are reported in the interpretive software do not modify the interpretations. If the QC for a plate fails, all the interpretations for that plate should be considered inconclusive.

NOTE: + = Detected, – = Not Detected, ? = Inconclusive

N1	N2	N501Y Assay Pair	E484K Assay Pair	K417N Assay Pair	K417T Assay Pair	L452R Assay Pair	Del69&70 Assay Pair	RNase P	Interpretation
Any 2 or more +									Detected
–	–	–	–	–	–	–	–	+	Not Detected
–	–	–	–	–	–	–	–	–	No Template
Any other combination									Inconclusive

Output File Format

The interpretive output file format is a comma-separated values (.csv) file with the following information:

Line 1:	File format name and version
Line 2:	IFC barcode
Line 3:	Biomark scan date and time
Line 4:	Biomark system ID
Line 5:	Real-Time PCR Analysis software version
Lines 6–8:	Analysis parameters used by the Real-Time PCR Analysis software
Line 9:	Biomark Pathogen Detection Software version
Line 10:	Assay names
Lines 11–13:	Detected assay parameters used by the Biomark Pathogen Detection Software
Line 14:	Mutation assay names
Line 15:	Value of the delta Ct threshold for each assay
Line 17:	Controls for successful interpretation or errors, if encountering errors
Line 18:	(Headers) <ul style="list-style-type: none">Control Name: Name of the control entered in the Sample Setup (in the Real-Time PCR Analysis software)IFC Inlet Location: Sample inlet ID (IFC inlet map)Plate Name: Name of the sample platePlate Barcode: Barcode of the sample platePlate Position: Position of the sample in the plateN1: N1 assay resultN2: N2 assay resultRNase P: RNase P assay resultDel69&70: Del69&70 assay resultG22813T (K417N): K417N assay resultA22812C (K417T): K417T assay resultT22917G (L452R): L452R assay resultG23012A (E484K): E484K assay resultA23063T (N501Y): N501Y assay resultResult: Interpretation according to the assay results.
Lines 19–26:	Results for each control (per sample plate) NOTE: The number of lines needed for the control results depends on the number of controls that are defined. For example, if only 3 controls are defined (Lines 19–24), the following line numbers will shift accordingly (Lines 25, 26, 27–).
Line 28:	Samples for successful interpretation or errors, if encountering errors

Line 29:	(Headers)	
	<ul style="list-style-type: none"> • Sample Name: Name entered in the Sample Setup (in the Real-Time PCR Analysis software) • IFC Inlet Location: Sample inlet ID (IFC inlet map) • Plate Name: Name of the sample plate • Plate Barcode: Barcode of the sample plate • Plate Position: Position of the sample in the plate • N1: N1 assay result • N2: N2 assay result • RNase P: RNase P assay result • Del69&70 Wild Type: Del69&70 Wild Type assay result • K417N Wild Type: K417N Wild Type assay result • K417T Wild Type: K417T Wild Type assay result • L452R Wild Type: L452R Wild Type assay result • E484K Wild Type: E484K Wild Type assay result • N501Y Wild Type: N501Y Wild Type assay result • Del69&70 Mutant: Del69&70 Mutant assay result • K417N Mutant: K417N Mutant assay result • K417T Mutant: K417T Mutant assay result • L452R Mutant: L452R Mutant assay result • E484K Mutant: E484K Mutant assay result • N501Y Mutant: N501Y Mutant assay result • Del69&70 Delta Ct: Del69&70 Delta Ct value • K417N Delta Ct: K417N Delta Ct value • K417T Delta Ct: K417T Delta Ct value • L452R Delta Ct: L452R Delta Ct value • E484K Delta Ct: E484K Delta Ct value • N501Y Delta Ct: N501Y Delta Ct value • COVID-19 Interpretation: Interpretation according to the assay results. • Del69&70 Interpretation: Presence or absence of Del69&70 • G22813T (K417N) Interpretation: Presence or absence of K417N • A22812C (K417T) Interpretation: Presence or absence of K417T • T22917G (L452R) Interpretation: Presence or absence of L452R • G23012A (E484K) Interpretation: Presence or absence of E484K • A23063T (N501Y) Interpretation: Presence or absence of N501Y • Plate QC Result: Quality control results for the plate 	
Lines 30– :	Results for each sample	

Example of a Successful Output File for the Advanta SARS-CoV-2 Mutation Assay Panel, Rows 1–27

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Biomark Pathogen Detection Software Output (Rev B)															
2	IFC Barcode	1691330277														
3	Biomark Scan Date and Time	4/28/2021 14:15														
4	Biomark System ID	BIOMARKHD542														
5	Real-Time PCR Analysis Software Version	4.7.2														
6	Quality Threshold	0.65														
7	Baseline Correction Method	Linear														
8	Ct Threshold Method	Auto By Control (Global)														
9	Biomark Pathogen Detection Software Version	2.1.1														
10	Assays	N1	N2	RNase P	Del69&70 Wild Type	K417N Wild Type	K417T Wild Type	L452R Wild Type	E484K Wild Type	N501Y Wild Type	Del69&70 Mutant	K417N Mutant	K417T Mutant	L452R Mutant	E484K Mutant	N501Y Mutant
11	Maximum Detected Ct	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
12	Number of Detected Assay Replicates	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
13	Assay Detection Rules	Defined Detected	All Not Detected													
14	Rule Parameters	Del69&70	K417N	K417T	L452R	E484K	N501Y									
15	Delta Ct Threshold	6	6	6	6	6	6									
16																
17	Controls															
18	Control Name	IFC Inlet Location	Plate Name	Plate Barcode	Plate Position	N1	N2	RNase P	Del69&70 (K417N)	G22813T (K417N)	A22812C (K417T)	T22917G (L452R)	G23012A (E484K)	A23063T (N501Y)	Result	
19	NTC	S001	Plate 1		A1	-	-	-	No Template	No Template	No Template	No Template	No Template	No Template	No Template	Pass
20	NC	S025	Plate 1		B1	-	-	+	No Template	No Template	No Template	No Template	No Template	No Template	No Template	Pass
21	PC Wild Type	S049	Plate 1		C1	+	+	+	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Pass
22	PC Mutant	S073	Plate 1		D1	-	-	+	Present	Present	Present	Present	Present	Present	Present	Pass
23	NTC	S007	Plate 2		A1	-	-	-	No Template	No Template	No Template	No Template	No Template	No Template	No Template	Pass
24	NC	S031	Plate 2		B1	-	-	+	No Template	No Template	No Template	No Template	No Template	No Template	No Template	Pass
25	PC Wild Type	S055	Plate 2		C1	+	+	+	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Pass
26	PC Mutant	S079	Plate 2		D1	-	-	+	Present	Present	Present	Present	Present	Present	Present	Pass
27																

Example of a Successful Output File for the Advanta SARS-CoV-2 Mutation Assay Panel Rows 28–, Columns A–P

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
28	Samples															
29	Sample Name	IFC Inlet Location	Plate Name	Plate Barcode	Plate Position	N1	N2	RNase P	Del69&70 Wild Type	K417N Wild Type	K417T Wild Type	L452R Wild Type	E484K Wild Type	N501Y Wild Type	Del69&70 Mutant	K417N Mutant
30		0 S002	Plate 1		A2	-	-	+	-	-	-	-	-	-	-	-
31		50 S003	Plate 1		A3	+	+	+	+	+	+	+	+	-	-	-
32		50 S006	Plate 1		A6	+	+	+	+	+	+	+	+	-	-	-
33		50 S005	Plate 1		A5	+	+	+	+	+	+	+	-	-	-	-
34		50 S004	Plate 1		A4	+	+	+	+	+	+	+	-	-	-	-
35		50 S013	Plate 1		A7	+	+	+	+	+	+	+	-	-	-	+
36		50 S014	Plate 1		A8	+	+	+	+	+	+	+	-	+	-	-
37		50 S015	Plate 1		A9	+	+	+	+	+	+	+	-	-	-	-
38		50 S018	Plate 1		A12	+	+	+	+	+	+	+	+	-	-	-
39		50 S017	Plate 1		A11	+	+	+	+	+	+	+	-	-	-	-
40		50 S016	Plate 1		A10	+	+	+	+	+	+	+	-	-	-	-
41		0 S026	Plate 1		B2	-	-	+	-	-	-	-	-	-	-	-
42		25 S027	Plate 1		B3	+	+	+	+	+	+	+	-	-	-	-
43		25 S030	Plate 1		B6	+	+	+	+	+	+	+	-	-	-	-
44		25 S029	Plate 1		B5	+	+	+	+	+	-	+	-	-	-	-

Example of a Successful Output File for the Advanta SARS-CoV-2 Mutation Assay Panel Rows 28–, Columns Q–AH

	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
28																		
29	K417T Mutant	L452R Mutant	E484K Mutant	N501Y Mutant	Del69&70 Delta Ct	K417N Delta Ct	K417T Delta Ct	L452R Delta Ct	E484K Delta Ct	N501Y Delta Ct	COVID-19 Interpretation	Del69&70 Interpretation	G22813T (K417N) Interpretation	A22812C (K417T) Interpretation	T22917G (L452R) Interpretation	G23012A (E484K) Interpretation	A23063T (N501Y) Interpretation	Plate QC Result
30	-	-	-	-	NA	NA	NA	NA	NA	NA	Not Detected	No Template	No Template	No Template	No Template	No Template	No Template	Pass
31	+	-	+	+	999	999	-10.5	999	-9.74	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
32	+	-	+	+	999	14.58	-11.21	13.43	-9.37	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
33	+	-	+	+	999	999	-9.93	999	-999	-12.05	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
34	+	-	+	+	999	999	-10.62	999	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
35	+	-	+	+	999	10.47	-10.91	13.49	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
36	+	-	+	+	999	999	-10.2	999	-999	-6.87	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
37	+	-	+	+	15.46	999	-10.03	999	-15.1	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
38	+	-	+	+	999	999	-11.11	999	-8.65	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
39	+	-	+	+	999	999	-10.8	12.32	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
40	+	-	+	+	999	999	-11.18	999	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
41	-	-	-	-	NA	NA	NA	NA	NA	NA	Not Detected	No Template	No Template	No Template	No Template	No Template	No Template	Pass
42	+	-	+	+	999	999	-10.65	999	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
43	+	-	+	+	999	999	-10.31	999	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass
44	+	-	+	+	999	999	-12.12	999	-999	-999	Detected	Absent	Absent	Present	Absent	Present	Present	Pass

Example of an Error

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Biomark Pathogen Detection Software Output (Rev B)															
2	IFC Barcode	1691271009														
3	Biomark Scan Date and Time	4/1/2021 19:49														
4	Biomark System ID	BIOMARKHD081														
5	Real-Time PCR Analysis Software Version	4.7.1														
6	Quality Threshold	0.65														
7	Baseline Correction Method	Linear														
8	Ct Threshold Method	Auto By Control (Global)														
9	Biomark Pathogen Detection Software Version	2.1.1														
10	Assays	N1	N2	RNase P	Del69&70 Wild Type	K417N Wild Type	K417T Wild Type	L452R Wild Type	E484K Wild Type	N501Y Wild Type	Del69&70 Mutant	K417N Mutant	K417T Mutant	L452R Mutant	E484K Mutant	N501Y Mutant
11	Maximum Detected Ct	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
12	Number of Detected Assay Replicates	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1
13	Assay Detection Rules	Defined Detected	All Not Detected													
14	Rule Parameters	Del69&70	K417N	K417T	L452R	E484K	N501Y									
15	Delta Ct Threshold	4	4	4	4	4	4									
16																
17	Errors															
18	The input data file doesn't contain the following interpretive assays	N501Y Wild Type	N501Y Mutant													

Appendix A: Reporting Ct Values

You can configure the Biomark Pathogen Detection Software to include the Ct values in the output file for detected assay replicates by entering **-ct** in the Additional Arguments field or at the end of the command line.

NOTE: If you are entering **-ct** after **-re**, separate the 2 arguments with a space (**-re -ct**).

In the output file, the number and average Ct of detected assay replicates appear after the Results column in the Control Name row (Line 18) and after the Plate QC Results column in the Sample Name row (Line 29, if using 4 controls):

Control Name row (headers)

- N1 # of Detected Assay Replicates
- N2 # of Detected Assay Replicates
- RNase P # of Detected Assay Replicates
- N1 Average Detected Ct
- N2 Average Detected Ct
- RNase P Average Detected Ct

Sample Name row (headers)

- N1 # of Detected Assay Replicates
- N2 # of Detected Assay Replicates
- RNase P # of Detected Assay Replicates
- Del69&70 Wild Type # of Detected Assay Replicates
- K417N Wild Type # of Detected Assay Replicates
- K417T Wild Type # of Detected Assay Replicates
- L452R Wild Type # of Detected Assay Replicates
- E484K Wild Type # of Detected Assay Replicates
- N501Y Wild Type # of Detected Assay Replicates
- Del69&70 Mutant # of Detected Assay Replicates
- K417T Mutant # of Detected Assay Replicates
- K417N Mutant # of Detected Assay Replicates
- L452R Mutant # of Detected Assay Replicates
- E484K Mutant # of Detected Assay Replicates
- N501Y Mutant # of Detected Assay Replicates
- N1 Average Detected Ct
- N2 Average Detected Ct
- RNase P Average Detected Ct
- Del69&70 Wild Type Average Detected Ct
- K417N Wild Type Average Detected Ct
- K417T Wild Type Average Detected Ct
- L452R Wild Type Average Detected Ct
- E484K Wild Type Average Detected Ct

- N501Y Wild Type Average Detected Ct
- Del69&70 Mutant Average Detected Ct
- K417N Mutant Average Detected Ct
- K417T Mutant Average Detected Ct
- L452R Mutant Average Detected Ct
- E484K Mutant Average Detected Ct
- N501Y Mutant Average Detected Ct

NOTE: The Ct values are only reported for positive results. Negative results are not reported (NA).

Appendix B: Mutation Assays

Product Name	Nucleotide Position	WT Nucleotide	Mutation	AA Change	Found in [Strain]
K417T Wild Type, K417T Mutant	22812	A	C	K417T	Brazil P1
K417N Wild Type, K417N Mutant	22813	G	T	K417N	SA B.1.351
L452R Wild Type, L452R Mutant	22917	T	G	L452R	CA B.1.427, B.1.429
E484K Wild Type, E484K Mutant	23012	G	A	E484K	SA B.1.351; Brazil P1; NY B.1.526
N501Y Wild Type, N501Y Mutant	23063	A	T	N501Y	UK B.1.1.7; SA B.1.351; BrazilP1
Del69&70 Wild Type, Del69&70 Mutant	Δ69/70	—	—	—	UK B.1.1.7

For technical support visit techsupport.fluidigm.com.

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