TaqMan™ 2019-nCoV Assay Kit v1

For use with TaqMan™ 2019-nCoV Control Kit v1 for pathogen detection of SARS-CoV-2 RNA

Catalog Number A47532

Pub. No. MAN0019096 Rev. A.0

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Applied Biosystems™ TaqMan™ 2019-nCoV Assay Kit v1 (Cat. No. A47532) contains a set of TaqMan™ RT-PCR assays for the qualitative detection and characterization of SARS-CoV-2 RNA. The kit includes three assays that target SARS-CoV-2 genes, and one positive control assay that targets the Human RNase P RPPH1 gene:

- Assays target three different viral genomic regions, reducing the risk of false negatives.
- Assays have undergone bioinformatic selection and analysis to specifically target sequences that are unique to SARS-CoV-2.
- The RNase P assay is run in duplex with each 2019-nCoV assay as an internal positive control.
- The 2019-nCoV assays are designed to work with total nucleic acid that is isolated from research samples collected via nasopharyngeal swab, nasopharyngeal aspirate, and bronchoalveolar lavage (BAL).

The kit is used with the TaqMan™ 2019-nCoV Control Kit v1 (Cat. No. A47533) to monitor assay-specific amplification.

For more information about genetic analysis solutions available for SARS-CoV-2, go to **thermofisher.com/coronavirus**.

Contents and storage

Contents	Dye	Amount[1]	Concentration	Storage
2019-nCoV (ORF1ab) (Tube 1)	FAM™ dye	75 µL	20X	
2019-nCoV (S Protein) (Tube 2)	FAM™ dye	75 µL	20X	–30°C to –10°C
2019-nCoV (N Protein) (Tube 3)	FAM™ dye	75 µL	20X	
RNase P Assay (Tube 4)	VIC™ dye	250 µL	20X	

[1] Sufficient for 50 × 25-µL reactions.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source
Real-Time PCR Instrument (one of the following)	
QuantStudio™ 5 Real-Time PCR System (96-well, 0.2-mL Block)	Contact your local sales office
7500 Real-Time PCR Instrument	
Real-Time PCR Instrument Software (one of the following)[1]	
QuantStudio™ Design and Analysis Software v1.5.1	Included with QuantStudio™ 5 Real-Time PCR System
7500 Software v2.3	Included with 7500 Real-Time PCR Instrument

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Item	Source
Reagents	
TaqMan™ 2019-nCoV Control Kit v1	A47533
Master Mix (one of the following)[2]: • TaqPath™ 1-Step RT-qPCR Master Mix, CG (contains dUTP/UNG) • TaqMan™ Fast Virus 1-Step Master Mix	• A15299, A15300 • 4444432, 4444434, 4444436
Tubes, plates, and other consumables	
MicroAmp™ Optical 96-Well Reaction Plate (0.2-mL)	N8010560, or equivalent[3]
MicroAmp™ Optical Adhesive Film	4311971, 4360954
Data Analysis Software (one of the following)[4]	
Design and Analysis Software v2.3	**thermofisher.com/qpcrsoftware**
ExpressionSuite™ Software v1.3	**thermofisher.com/expressionsuite**

[1] Real-Time PCR Instrument Software is used to control the instrument and to collect instrument run data.

[2] Other Master Mixes are supported, but have not been tested.

[3] See **thermofisher.com/plastics**.

[4] We recommend using either Design and Analysis Software v2.3 or ExpressionSuite™ Software v1.3 for data analysis because these softwares use updated algorithms with improved sensitivity for detection of low copy samples.

Procedural guidelines

- Use purified, non-degraded total nucleic acid that is free of RNase activity and RT-PCR inhibitors.

  Note: To perform nucleic acid isolation using the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit, see "Related documentation".

- Protect the assays and master mix from light.

- To avoid false negatives, test each research sample with all three of the 2019-nCoV assays.

- For each reaction, run the 2019-nCoV assay in duplex with the RNase P assay.

- Before you begin, determine the number of required reactions. In addition to the nucleic acid research samples, include the following reactions for each 2019-nCoV assay:
  - One 2019-nCoV Control v1 reaction per 2019-nCoV assay per plate
  - One no-template control (NTC) per 2019-nCoV assay per plate

Perform 1-Step RT-PCR

1. For each 2019-nCoV assay, combine the following components for the number of reactions, plus 10% overage:

Component	Volume per reaction
Master Mix (4X)	6.25 µL
2019-nCoV assay (20X)	1.25 µL
RNAse P assay (20X)	1.25 µL
RT-PCR Grade Water	11.25 µL
Total Reaction Mix volume	**20.0 µL**

2. For each reaction, combine the following components in a MicroAmp™ Optical 96-Well Reaction Plate (0.2-mL) well.

Component	Volume per well
Reaction Mix (see step 1)	20.0 µL
• Nucleic acid research sample *or* • 1 µL 2019-nCoV Control v1 + 4 µL RT-PCR Grade Water *or* • NTC	5.0 µL
Total reaction volume	**25.0 µL**

3. Set up and run the reactions on a real-time PCR instrument using the following settings:
   - Analysis method: Comparative C_t

     Note: You must use Comparative C_t to analyze 2019-nCoV assay data using Design and Analysis Software v2 and ExpressionSuite™ Software.
   - Cycling mode: Standard
   - Thermal protocol:

Stage	Step	Temperature	Time	
			TaqPath™ 1-Step RT-qPCR Master Mix, CG	TaqMan™ Fast Virus 1-Step Master Mix
Hold	UNG incubation[1]	25°C	2 minutes	N/A[2]
Hold	Reverse transcription	50°C	15 minutes	5 minutes
Hold	Activation[3]	95°C	2 minutes	20 seconds
Cycling (40 cycles)	Denaturation	95°C	3 seconds	3 seconds
	Anneal/Extension	60°C	30 seconds	30 seconds

[1] Heat-labile UNG in TaqPath™ 1-Step RT-qPCR Master Mix, CG is completely inactivated during the first ramp to 95°C.
[2] TaqMan™ Fast Virus 1-Step Master Mix does not include UNG.
[3] Required for RT inactivation, first denaturation, and activation of the DNA polymerase.

Analyze data

We recommend using either Design and Analysis Software v2.3 or ExpressionSuite™ Software v1.3 for data analysis because these programs have updated algorithms with improved sensitivity for detecting low-copy samples. For more information about using a software, see the software user guide or help.

Note: Design and Analysis Software v2 reports C_q values instead C_t values. The C_q values are equivalent to the C_t values indicated for data analysis and interpretation.

1. Open the data file (EDS) in the data analysis software.

   Note: Design and Analysis Software v2 requires data files created on a QuantStudio™ 5 Real-Time PCR System or 7500 Real-Time PCR Instrument to be saved as a new data file. Click **Actions ▸ Save As...**, then save the data file with a new name.

2. Perform analysis using **Auto Baseline** and **Auto Threshold** analysis settings.

3. For each plate, confirm that the control reactions for each 2019-nCoV assay perform as expected.

   Table 1 Expected results of the control reactions for each 2019-nCoV assay

Reaction	Expected C_t value	About unexpected results
NTC	Undetermined	If the NTC has an amplification curve that crosses the threshold (false positive), sample contamination may have occurred. Repeat the test with new reagents, following good RT-PCR practices.
2019-nCoV Control v1	$C_t \leq 30$	If the C_t value is above the expected result, repeat the test with new reagents.

4. Export the results.

If a positive control is not included on each plate, or a test sample has a $C_t \leq 15$, see "Additional guidelines for data analysis" on page 4.

Review results

1. Classify the results for each individual assay, according to the C_t values in the table:

Table 2   Individual assay results

2019-nCoV assay (FAM™ dye)	RNAseP assay (IPC) (VIC™ dye)	2019-nCoV assay result
$C_t < 37$	Any value	Positive.
$37 \leq C_t < 40$	Any value	Inconclusive. Repeat the test. Once the test is repeated, the result for the assay is positive if $C_t < 37$ or if the result is consistent with first test result of $37 \leq C_t < 40$.
C_t = Undetermined *or* C_t =40	$C_t < 40$	Negative.
C_t = Undetermined *or* C_t =40	C_t = Undetermined *or* C_t =40	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

2. For each test sample, interpret the results using the table. We recommend that each lab do accuracy testing with appropriate samples to establish guidelines for interpreting results.

Table 3   2019-nCoV assay results interpretation

2019-nCoV assay results	Interpretation of results
Any two of the three assays are positive.	SARS-CoV-2 RNA is present.
Any one of the assays is positive in two different samples collected from the same research subject.	SARS-CoV-2 RNA is present.
All three of the assays are negative.	SARS-CoV-2 RNA is not present.

Additional guidelines for data analysis

- If a test sample has a $C_t \leq 15$, then the Auto Threshold level can be set too high to detect SARS-CoV-2 RNA in low-copy test samples. Review all of the results for the 2019-nCoV assay to ensure that all positive results are detected.
    a. In the Amplification Plot, review the baseline settings. If the Baseline End is < 39 and the C_t is "Undetermined", the Auto Threshold may be set too high.
    b. Evaluate the overall shape of the amplification curves. A sigmoidal amplification curve indicates true amplification.
    c. If needed, omit test sample wells with $C_t \leq 15$, then reanalyze the data to reset the Automatic Threshold at a level suitable for detection in the remaining wells.
- If a positive control for each 2019-nCoV assay is not included on a plate, then the software may set the Auto Threshold too low. To avoid false-positive calls, review all positive results for the plate.
    a. For any well with a C_t value, examine the overall shape of the amplification curve.
    b. If the amplification curve does not have the expected sigmoidal shape, confirm amplification using another method:

Table 4   Review results: plates without positive controls

Data Analysis Software	Action

Design and Analysis Software v2.3	For each well, review the **Amp Status** to determine if true amplification occurred and the results are correct.
	  – **Amp**—Amplification occurred.
	  – **No Amp** or **Inconclusive**—Amplification has not occurred. Repeat the test.

ExpressionSuite™ Software v1.3	In the project that includes the test sample plates without positive controls, add a plate that includes a positive control reaction for each 2019-nCoV assay. Reanalyze the data to set the Automatic Threshold for all plates in the project.

Related documentation

Document	Publication Number
TaqMan™ 2019-nCoV Control Kit v1 Product Information Sheet	MAN0019097
TaqMan™ Gene Expression Assays User Guide—single-tube assays	4333458
MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (automated extraction) User Guide	MAN0018073

Limited product warranty

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