GenomeCoV19 Detection Kit

Store at -25 to -15°C

Cat. No.	Description	Quantity
G628	GenomeCoV19 Detection Kit	100 Rxns

For in vitro diagnostic use only. For professional use only.

Product Description

abm's GenomeCoV19 Detection Kit is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test intended for the qualitative detection of SARS-CoV-2 nucleic acid in human nasopharyngeal and oropharyngeal swab specimens.

- Highly specific for the RdRP and N targets recommended by WHO and US CDC
- Results in less than 2 hours
- Compatible with standard RT-qPCR machines (Bio-Rad's CFX96, QuantStudio's 7 Flex System)

Principle

The GenomeCoV19 Detection Kit uses real time PCR fluorescence technology to specifically detect RdRP and N genes from SARS-CoV-2 in human nasopharyngeal and oropharyngeal specimens. During the amplification process, the included probes will anneal to the specific target sequence located between the forward and reverse primers. The probe is then cleaved, releasing the reporter dye and generating a fluorescent signal. An internal control primer and probe set (RP) is included to monitor proper specimen collection and assay setup.

Kit Components

Product Component	Volume	Rxns per kit	Part No.
COVID-19 Primers/Probes	200 µl	100 X	G628-1
2X RT-qPCR MasterMix	1.25 ml	100 X	G628-2
Positive Control Template	100 µl	20 X	G628-3
Negative Extraction Control	1.0 ml	20 X	G628-4
RT-qPCR Enzyme Mix	40 µl	100 X	RT-13
Nuclease-free H_2O	1.0 ml	100 X	RT-0

Storage and Stability

Upon arrival, store the kit components at -25 to -15°C for up to 12 months.

Sample Collection, Storage and Transport

- Applicable sample types: nasopharyngeal and oropharyngeal swabs
- Flocked swabs are preferred. Sterile dacron or rayon swabs with plastic or flexible metal handles may also be used. Do NOT use cotton or calcium alginate swabs or swabs with wooden sticks as they may contain substances that inactivate viruses and inhibit PCR.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.
- It is recommended to use **abm**'s Sample Collection and Viral Transport Solution (Cat. No. G631).

Sample Preparation

We recommend the use of Qiagen QIAamp DSP Viral RNA Mini Kit (#61904) for RNA extraction. Please follow the manufacturer's instructions. Precautions must be taken to prevent cross-contamination of samples. To monitor that there is no cross -contamination during the extraction process, extract the Negative Extraction Control (G628-4) included in this kit alongside your samples for each sample preparation run. Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or at -70°C for long term storage. Separate work areas should be used for nucleic acid extraction and reagent preparation.

Protocol

Proper microbiological, aseptic technique should always be followed when working with RNA. Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from laboratory equipment. During the procedure, work quickly and keep all reagents on cold blocks when possible to avoid degradation of RNA.

1. *RT-qPCR MasterMix Preparation:* Prepare sufficient quantity of the following reagent mix for the number of samples and controls being tested:

Reagent	Volume per reaction
COVID-19 Primers/Probes	2 µl
2X RT-qPCR MasterMix	10 µl
RT-qPCR Enzyme Mix	0.4 µl
Nuclease-free H_2O	2.6 µl

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2. In PCR clean room or BSL2 Biosafety hood, add 15 µl of the RT-qPCR MasterMix prepared in Step 1 to required wells of PCR plate.
3. Add 5 µl of nuclease-free H_2O to the negative control well and cap accordingly. This is the no-template-control (NTC) reaction.
4. Move the PCR plate to Template Addition Room.
5. Add 5 µl of extracted nucleic acid from each patient sample to the test wells.
6. Add 5 µl of extracted nucleic acid from Negative Extraction Control to the negative extraction control well.
7. Add 5 µl of Positive Control Template to the positive control well.
8. Cap all wells securely with optical caps or seal the plate with an optical film.
9. Centrifuge the PCR plate to collect all liquid in the bottom of the wells using a tabletop refrigerated centrifuge.
10. Transfer the PCR plate to a qPCR instrument.

Standard RT-qPCR Cycling Conditions

Transfer the reaction setup into a qPCR machine and set up the following cycling program. It is recommended to use BioRad's CFX96, or QuantStudio's 7 Flex system.

Steps	Temperature	Time	Cycle(s)
cDNA Synthesis	42°C	15 minutes	1
Pre-Denaturation	95°C	10 minutes	1
Denaturation	95°C	15 seconds	40
Annealing	60°C	60 seconds	

Detection Channels

Three channels (FAM, HEX and ROX) are used in this single tube qPCR assay. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer. Select "None" for ROX passive reference on any qPCR machines requiring ROX as reference dye.

Expected Performance of Controls

Control Type	Used to Monitor	Expected Results and Ct Values		
		N (FAM)	RdRP (ROX)	RP (HEX)
Negative ("NTC")	Assay or extraction reagent contamination	Negative Ct ND	Negative Ct ND	Negative Ct ND
Positive	Improper assay setup and reagent failure, including primer and probe degradation	Positive Ct < 40.0	Positive Ct < 40.0	Negative Ct ND
Negative Extraction Control	Cross-contamination during extraction	Negative Ct ND	Negative Ct ND	Positive Ct < 40.0
Positive Extraction Control ("RP")	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, or PCR inhibition	Negative Ct ND	Negative Ct ND	Positive Ct < 40.0

ND = Not Detected. If any control does not perform as specified above, results are considered invalid.

Interpretation of Results

SARS-CoV-2			Interpretation	Action
N	RdRP	RP		
+	+	+/-	Positive	Report result to sender health authority.
If only one of the two targets are positive		+/-	Inconclusive Result	Repeat RT-qPCR of samples or repeat from extraction step. If result is still inconclusive, recommend collection of new specimen(s) from the patient.
-	-	+	Negative	SARS-CoV-2 not detected. Report result to sender health authority
-	-	-	Invalid Result	Repeat from extraction step. If the repeated result remains invalid, recommend collection of a new specimen(s) from the patient.

Limitation of Test Methods

The test results of this kit are only for clinical reference. Clinical diagnosis and treatment of sick patients should be considered in combination with their symptoms/signs, medical history and results of other laboratory examinations.

Possible causes for false negative results:

- Improper sample collection, transportation and treatment, and/or excessively low virus droplets in samples.
- Mutations in the target sequence of SARS-CoV-2 or changes in the sequence caused by other reasons.
- Other untested interferences or PCR inhibitors.

False positive results may occur if cross-contamination is not well managed during sample processing.

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Performance Characteristics

Limit of Detection (LoD): Positive reference sample was tested using both positive control template (IDT, Cat 10006625) and in vitro transcribed RNA control (IVT-RNA) from in house. The initial LoD was determined by testing template at 5000, 500, 50 and 5 cp/ reaction in quadruplicate. The final LoD was confirmed by testing additional 20 replicates of samples at 5 cp/reaction (20/20 positive results). At the LoD threshold, the Ct was observed to have a mean of 32.72 (SD 0.43, % CV 1.32).

Clinical Performance: A contrived clinical study was conducted to evaluate the clinical performance. A total of 60 individual nasopharyngeal clinical specimens, collected from volunteers and presumed negative for SARS-CoV-2, were used in this study. A total of 30 negative and 30 contrived positive samples were tested. Positive samples were prepared by spiking BEI ATCC Genomic RNA from SARS Related Coronavirus 2 (Catalog No. NR-52285) into NP matrix mixed with lysis buffer from QIAamp DSP Viral RNA Mini Kit at 1X, 10X, and 100X LoD. Results showed 100% Positive Percent Agreement (30/30 positive results) and 100% Negative Percent Agreement (30/30 negative results).

Inclusivity (Analytical Sensitivity): The primers and probes sequences were blasted against SARS-CoV-2 genomes publicly available as of on March 20th, 2020. Results showed the sequences had 100% homology to all SARS-CoV-2 isolates analyzed, with two exceptions of MT159720.1 and MT258382.1 (homology of 94% and 95% respectively). Both exceptions occur at the 5'end of the primer, and thus are unlikely to cause the failure of qPCR, and would not affect the test performance under specified annealing temperature.

Cross-Reactivity: The detection results of this kit displayed no cross-reactivity with influenza A virus, influenza B virus, adenovirus, *Staphylococcus epidermis*, HCoV-SARS, HCoV-OC43, HCoV-HKU1, or *Streptococcus pneumonia*.

Precision: CV < 5% (Between and within batches).

Precautions

- Any personnel performing the experiment must be professionally trained.
- Clinical samples should be regarded as potentially infectious materials and should be handled in a Biological Safety Cabinet.
- This assay needs to be run according to Good Laboratory Practice guidelines.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of reagents, as this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge the tubes briefly before use.
- Quickly prepare the Reaction Mix on ice or in the cooling block.
- Each process in the experiment should be conducted in different designated zones (reagent preparation zone, sample processing zone, amplification zone and product analysis zone).
- Pipettes, vials and other working materials should not be circulated among different working zones.
- Always use sterile pipette tips with filters.

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