GenomeCoV19 Detection Kit

Store at -25 to -15°C

Cat. No.	Description	Quantity
G628.v2	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 RNA from SARS-CoV-2 in human nasopharyngeal and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information. This can include the patient's recent exposures, history, or presence of clinical signs and symptoms consistent with COVID-19.

The GenomeCoV19 Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. Validation of this kit has not been reviewed by the FDA. Review under the EUA program is pending. This kit is distributed in accordance with the guidance on Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised), Section IV.C.2.

Key features of the GenomeCoV19 Detection Kit include:

- Highly specific for the S and N targets
- Results in less than 2 hours
- Compatible with the Bio-Rad's CFX96 qPCR machine

Principle

The GenomeCoV19 Detection Kit uses real time PCR fluorescence technology to specifically detect S 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 (Actin) 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.v2
2X RT-qPCR MasterMix	1.25 ml	100 X	G628-2
Positive Control Template	100 µl	20 X	G628-3.v2
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

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.

Steps	Temperature	Time	Cycle(s)
cDNA Synthesis	50°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 CY5) are used in this single tube qPCR assay. It is recommended to perform the color (channel) calibration as requested by the instrument's manufacturer.

Expected Performance of Controls

Control Type	Used to Monitor	Expected Results and Ct Values		
		N (FAM)	S (HEX)	Actin (CY5)
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 ("Actin")	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	S	Actin		
+	+	+/-	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

Please note that the GenomeCoV19 Detection Kit has been validated in accordance with the FDA's Emergency Use Authorization guidance, however, the FDA's independent review of this validation is still pending. Additionally, laboratories using the GenomeCoV19 Detection Kit should include the statement "This test has been validated but the FDA's independent review of this validation is still pending" with patient test reports provided to their healthcare providers.

The test results of this kit are 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.

Performance Characteristics

Limit of Detection (LoD): Our LoD study established the lowest concentration of SARS-CoV-2 RNA, as genome copies (cp)/reaction, which can be detected by the GenomeCoV19 Detection Kit at least 95% of the time. This was established by testing serial dilutions of SARS-CoV-2 genomic RNA (source: ATCC-VR1986D) into nasopharyngeal clinical matrix, verified to be COVID-19 negative, at the following concentrations: 100 cp/μL, 50 cp/μL, 5 cp/μL, 4 cp/μL, 3 cp/μL, 2 cp/μL, 1 cp/μL and 0.5 cp/μL. RNA extraction was performed with the Qiagen QIAamp DSP Viral RNA Mini Kit and tests were run on the Bio-Rad CFX96 qPCR instrument. The LoD was determined to be 5 cp/reaction and results are shown in the below.

Cp/Reaction	Samples Tested	N-gene (FAM)	Average Ct (FAM)	S-gene (HEX)	Average Ct (HEX)	% Match
500	4	4	27.29	4	27.75	100%
250	4	4	28.35	4	28.84	100%
25	4	4	31.65	4	32.19	100%
20	4	4	31.33	4	31.10	100%
15	4	4	31.72	4	31.25	100%
10	4	4	32.64	4	32.24	100%
5	24	24	32.99	24	33.36	100%
2.5	4	3	37.15	4	36.12	75%

Clinical Performance (Contrived Samples): A contrived clinical study was conducted with a total of 30 negative and 30 contrived positive samples. Positive samples were prepared by spiking ATCC-VR1986D SARS-CoV-2 reference genome RNA into nasopharyngeal matrix mixed with lysis buffer from QIAamp DSP Viral RNA Mini Kit at 1X, 10X, and 100X LoD. Tests were run on the Bio-Rad CFX96 qPCR instrument and results showed 100% Positive Percent Agreement (30/30 positive results) and 100% Negative Percent Agreement (30/30 negative results).

Clinical Performance (Remnant Samples): A blinded clinical study was conducted using a SARS-CoV-2 Validation Panel with 60 nasopharyngeal/oropharyngeal/nasal swab remnant clinical specimens. These 30 SARS-CoV-2 positive and 30 SARS-CoV-2 negative remnant specimens were verified using a FDA EUA-authorized COVID-19 detection kit. RNA extraction was performed with the Qiagen QIAamp DSP Viral RNA Mini Kit and tests were run on the Bio-Rad CFX96 qPCR instrument. GenomeCoV19 Detection Kit test 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 June 29th, 2020. Results showed the sequences had 100% homology to all SARS-CoV-2 isolates analyzed, with two exceptions of MT451113.1 and MT534319.1 (homology of 95.2% in both cases). 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: Cross-reactivity studies using a panel of organisms were conducted by both *in silico* analysis and wet-testing of whole organisms or purified nucleic acids. The GenomeCoV19 Detection Kit primers and probes were first analyzed *in silico* against each of the organisms listed in the table below using BLASTn. No cross-reactivity with >80% homology was found for any of the primer and probe sets. Additionally, the bacteria, viruses and pooled human wash described in the table below were included for wet-testing. Concentrations of 10^6CFU/mL (for bacteria) and 10^5pfu/mL (for viruses) were used to purify the nucleic acids using the Qiagen QIAamp DSP Viral RNA Mini Kit. There was no cross-reactivity observed for any of the organisms tested using the GenomeCoV19 Detection Kit on Bio-Rad's CFX96 qPCR instrument. Cross-reactivity was defined as Ct < 40.

Organism Name	N-gene	S-gene	Source
Human Coronavirus-229E	ND	ND	ATCC VR-740
Human Coronavirus-OC43	ND	ND	Zeptometrix NATCOV(OC43)-ST
Human Coronavirus-HKU1	ND	ND	ATCC VR-3262SD
Human Coronavirus-NL63	ND	ND	ATCC VR-3263SD
SARS-coronavirus	ND	ND	BEI NR-3882
MERS-coronavirus	ND	ND	ATCC VR-3248SD
Adenovirus	ND	ND	abm 000047A
Human Metapneumovirus	ND	ND	ATCC VR-3250SD
Parainfluenza virus 1	ND	ND	ATCC VR-94D; C35
Parainfluenza virus 2	ND	ND	ATCC VR-92D; Greer
Parainfluenza virus 3	ND	ND	ATCC VR-1782; ATCC-2011-5
Parainfluenza virus 4	ND	ND	ATCC VR-1377; CH 19503
Influenza A	ND	ND	ATCC VR-1679D; H3N2, A/Hong Kong/8/68
Influenza B	ND	ND	ATCC VR-1735D; B/Taiwan/2/62
Enterovirus	ND	ND	ATCC VR-1377; CH 19503
Respiratory syncytial virus	ND	ND	ATCC VR-1580; 18537
Rhinovirus	ND	ND	ATCC VR-1171; 6669-CV39
Chlamydia pneumoniae	ND	ND	ATCC 53592D; AR-39
Haemophilus influenzae	ND	ND	ATCC 51907D
Legionella pneumophila	ND	ND	ATCC 33152D-5; Philadelphia-1
Mycobacterium tuberculosis	ND	ND	ATCC 25177; H37Ra
Pneumocystis jirovecii	ND	ND	Zeptometrix- 0801698DNA-1UG
Streptococcus pneumoniae	ND	ND	ATCC 33400D-5
Streptococcus pyogenes	ND	ND	ATCC 12344D-5; T1
Streptococcus salivarius	ND	ND	Zeptometrix - 0801896
Mycoplasma pneumoniae	ND	ND	ATCC 15531D; FH of Eaton Agent
Pooled human nasal wash - representing diverse microbial flora in the human respiratory tract	ND	ND	-
Candida albicans	ND	ND	ATCC 10231
Bordetella pertussis	ND	ND	Zeptometrix 0801459
Pseudomonas aeruginosa	ND	ND	ATCC 9027
Staphylococcus epidermis	ND	ND	ATCC 12228

*ND = Not Detected

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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Index of Symbols

IVD	*In Vitro* Diagnostic Use		See Instructions for Use		Caution
REF	Catalog Number		Manufacturing Date	LOT	Lot Number
	Expiry Date	Σ	Number of Tests		Store at -15 ~ -25°C
	Manufacturer	CE	CE Marking	EC REP	European Authorized Representative

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