KimForest SARS-CoV-2 Detection Kit v1

CE

REF **Catalog number** KF2019CoV01 (96 tests)

Instructions for Use

For In Vitro Diagnostic Use Only

For Prescription Use Only

For Use Under Emergency Use Authorization Only

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INTENDED USE

The KimForest SARS-CoV-2 Detection Kit v1 is a real-time reverse transcription-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs, anterior/mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, and bronchoalveolar lavage (BAL) 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, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA which is generally detected in 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 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.

Testing with the KimForest SARS-CoV-2 Detection Kit v1 is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays and in vitro diagnostic procedures. The KimForest SARS-CoV-2 Detection Kit v1 is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

SUMMARY and EXPLANATION

In December 2019, severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) was confirmed to cause severe pneumonia in humans. This infectious disease has been announced as a global pandemic by the World Health Organization (WHO). Common signs of infection include respiratory symptoms, fever, cough, shortness of breath and breathing difficulties. In severe cases, infection can cause pneumonia, acute respiratory distress syndrome (ARDS), kidney failure and death.

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The 2019 Novel Coronavirus, formerly known as 2019-nCoV and now known as SARS-CoV-2, is a new strain of coronavirus that was first identified during the 2019-2020 pandemic.

SPECIMENS COLLECTING, TRANSPORTING and STORING

Collecting the Specimen

- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Follow specimen collection device manufacturer instructions for proper collection methods.

- Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 1-3 ml of appropriate transport media, such as viral transport media (VTM).
- Bronchoalveolar lavage specimens should be collected into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Transporting Specimens

- Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to CDC on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to CDC on dry ice.

Storing Specimens

- Specimens can be stored at 2-8°C for up to 72 hours after collection if needed.
- If a delay in extraction or shipping is expected, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at -70°C or lower.

PRINCIPLES of the PROCEDURE

KimForest SARS-CoV-2 Detection Kit v1 is designed based on TaqMan probe real-time RT-PCR technology. This kit includes assay primers/probes specific to the SARS-CoV-2 RdRp gene and the human Ribonuclease P (RNase P) gene as an endogenous control to monitor specimen collection, nucleic acid extraction and PCR amplification. KimForest SARS-CoV-2 Detection Kit v1 contains a positive control (PC) and a non-template control (NTC) that must be run in all experiments to assess for potential false negative/positive results. The master mix solution contains the enzymes needed to perform reverse transcription and real-time PCR. The KimForest SARS-CoV-2 Detection Kit v1 has sufficient reagents for 96 samples or controls.

REAGENTS and MATERIALS

Cont. **Materials provided with the KimForest SARS-CoV-2 Detection Kit v1**

Reagents	Description	Volume	Quantity	Storage
KF2019CoV-RdRp100 (20x)	RdRP (FAM)/hRNP (VIC) Assay	100 µl	96 tests	-25°C to -15°C
KFVirusMM (4x)	Real-time RT-PCR Master Mix	500 µl	96 tests	-25°C to -15°C
KF2019CoV-PC	2019CoV Positive Control	50 µl	10 tests	-25°C to -15°C
KF2019CoV-H20	Nuclease-Free Water	1 ml		-25°C to -15°C

Materials required but not provided

Qiagen QIAamp Viral RNA Mini Kit (Cat # 52904 for 50 preps and Cat # 52906 for 250 preps)

MicroAmp™ Optical 96-Well Reaction Plate

MicroAmp™ Optical Adhesive Film

MicroAmp™ Fast 8-Tube Strip, 0.1 mL

MicroAmp™ Optical 8-Cap Strips

Applied Biosystems StepOne/StepOnePlus Real-Time PCR Systems

SHIPMENT, STORAGE and STABILITY

- KimForest SARS-CoV-2 Detection Kit v1 is shipped and stored at -25℃ to -15℃ and should be kept away from light. If stored at the proper temperature, the expiration date on the printed labels should be adhered to.
- Repeated freezing and thawing should not exceed 3 cycles.
- If necessary, you could aliquot components into smaller volumes after resuspension for single-use.
- Do not use reagents past their expiration date.

QUALITY CONTROL

1. Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.
2. NTC (no template control): Each experiment must include an NTC to confirm that the reagents and environment have not been contaminated during the experiment. Please use KF2019CoV-H_2O in place of the extracted nucleic acid from the clinical sample.
3. PC (positive control): Each experiment must include a PC to monitor substantial reagent failure including primer and probe integrity. Please use KF2019CoV-PC in place of the extracted nucleic acid from the clinical sample. The positive control consists of a synthetic DNA plasmid harboring the RdRp gene of SARS-CoV-2 and the human RNaseP gene. The positive control is included with the KimForest SARS-CoV-2 Detection Kit v1 and is supplied at a concentration of 2.5X LoD.

WARNINGS and PRECAUTIONS ⚠

- **For in vitro diagnostic use (IVD) only.**
- **For Emergency Use Authorization only.**
- **For Prescription Use only.**
- **The KimForest SARS-CoV-2 Detection Kit v1 has not been FDA cleared or approved.**
- **The KimForest SARS-CoV-2 Detection Kit v1 has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.**
- **The KimForest SARS-CoV-2 Detection Kit v1 has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.**
- **The KimForest SARS-CoV-2 Detection Kit v1 is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.**
- **The operators should wear safety protective equipment and follow safety procedures set by your institution.**
- **All specimens should be considered as infectious agents. There are no known tests or methods that can completely rule out the specimens and their derived products are not infectious.**
- **These materials contain chemicals that may cause serious effects. Avoid splashing on eyes, skin or clothing.**
- **If it comes into contact with eyes, skin or mucous membranes, rinse immediately with plenty of water and seek medical treatment as soon as possible.**
- **After experiment, it is necessary to thoroughly clean the operation area.**
- **If the experiment is interrupted by any reason, it should be re-performed to meet the correct result.**

TEST PROCEDURE

Note: Determine the number of required reactions before starting the experiment. Besides the testing samples, please include the following experimental controls for each assay run:

- Positive control (PC) reaction using KF2019CoV-PC
- No template control (NTC) reaction using KF2019CoV-H_2O.

1. Combine the following components to prepare the reaction mix.
2. Determine the number of reactions (N). It is necessary to make excess reaction pre-mixture for the PC and NTC and for pipetting error. Use the following guide to determine N:

- If the number of testing samples (n) including experimental controls is less than 14, then N = n+1
- If the number of testing samples (n) including experimental controls is more than 14, then N = n+2

Reagents	Volume of reagent added per reaction
KF2019CoV-RdRp100 (20X)	N x 1 µl
KFVirusMM (4X)	N x 5 µl
KF2019CoV-H2O	N x 9 µl
Total reaction mix volume	N x 15 µl

3. Mix gently, spin down and transfer 15 µl of the reaction pre-mixture into a MicroAmp™ Fast Optical 96-Well Reaction Plate or MicroAmp™ Fast 8-Tube Strip, 0.1 mL.

4. Pipette 5 µl of extracted nucleic acid from each clinical sample, 5 µl KF2019CoV-PC, and 5 µl KF2019CoV-H_2O (serves as NTC) to each well for a final reaction volume of 20 µl.
5. Clinical samples, PC, and NTC should be added to the specific wells that are being tested as illustrated in **Figure 1**.

Figure 1. KimForest SARS-CoV-2 Detection Kit v1: An example for testing samples and experimental controls set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	RdRP S 1	RdRP S 9	RdRP S 17	RdRP S 25	RdRP S 33	RdRP S 41	RdRP S 49	RdRP S 57	RdRP S 65	RdRP S 73	RdRP S 81	RdRP S 89
B	RdRP S 2	RdRP S 10	RdRP S 18	RdRP S 26	RdRP S 34	RdRP S 42	RdRP S 50	RdRP S 58	RdRP S 66	RdRP S 74	RdRP S 82	RdRP S 90
C	RdRP S 3	RdRP S 11	RdRP S 19	RdRP S 27	RdRP S 35	RdRP S 43	RdRP S 51	RdRP S 59	RdRP S67	RdRP S 75	RdRP S 83	RdRP S92
D	RdRP S 4	RdRP S 12	RdRP S 20	RdRP S 28	RdRP S 36	RdRP S 44	RdRP S 52	RdRP S 60	RdRP S 68	RdRP S 76	RdRP S 84	RdRP S 92
E	RdRP S5	RdRP S 13	RdRP S 21	RdRP S 29	RdRP S 37	RdRP S 45	RdRP S 53	RdRP S 61	RdRP S 69	RdRP S 77	RdRP S 85	RdRP S 93
F	RdRP S6	RdRP S 14	RdRP S 22	RdRP S 30	RdRP S 38	RdRP S 46	RdRP S 54	RdRP S 62	RdRP S 70	RdRP S 78	RdRP S 86	RdRP S94
G	RdRP S 7	RdRP S 15	RdRP S 23	RdRP S 31	RdRP S 39	RdRP S 47	RdRP S 55	RdRP S 63	RdRP S 71	RdRP S 79	RdRP S 87	RdRP PC
H	RdRP S 8	RdRP S16	RdRP S 24	RdRP S 32	RdRP S 40	RdRP S 48	RdRP S 56	RdRP S 64	RdRP S 72	RdRP S 80	RdRP S 88	RdRP NTC

6. Seal plate using the Optical Adhesive Film or cover the 8-Tube Strips with the Optical 8-Cap Strips.
7. Centrifuge the plate or tubes.
8. Set up and start the experiment on the real-time PCR instrument using the following settings:
   - Analysis method: Quantitation-Relative Standard Curve
   - Cycling mode: Fast (approximately 40 minutes to complete an experiment)
   - Setting of Reporter and Quencher

Target	Reporter	Quencher
RdRp	FAM	NFQ (none)
RNase P	VIC	NFQ (none)
Passive Reference	ROX	NA

NFQ – Non-fluorescent quencher

- **Thermal protocol:**

Stage	Step	Temperature	Time
Hold	Reverse transcription	50°C	10 minutes
Hold	Activation	95°C	30 seconds
Cycling (40 cycles)	Denaturation	95°C	3 seconds
	Anneal	53°C	15 seconds
	Extension	60°C	15 seconds

RESULT INTERPRETATION

1. Open the data file using the data analysis software and perform analysis with auto threshold analysis setting.
2. For each experiment, confirm positive control (PC) and blank control (NTC) precautions for SARS-CoV-2 Detection Kit v1 as listed in **Table 1**.

Table 1. Expected Results for Each Experimental Control

Experimental controls	Used to monitor	RdRp gene (FAM)	RNase P gene (VIC)	Expected Ct values
Positive control (PC)	Substantial reagent failure including primer and probe integrity	+	+	Ct ≤ 38
Blank control (NTC)	Reagent and/or environmental contamination	-	-	Undetermined

Undetermined; No amplification

3. If any of the experimental controls do not meet the expected performance as described, the experiment may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the experiment.

   Note: Re-run the assay using residual nucleic acid for clinical samples and a new batch of controls.

4. Export the results.
5. The clinical sample results are interpreted according to the following criteria (**Table 2**):

Table 2. Testing Result Interpretation

RdRp gene	RNase P gene (IC)	Testing Result	Actions
Ct ≤ 38	Any value	Positive	Report testing results to public health authorities including CDC and sender.
Undetermined	Ct ≤ 38	Negative	Report results to sender. Testing for other respiratory virus should be considered.
38 < Ct ≤ 40	Ct ≤ 38	Inconclusive	Repeat the procedures of nucleic acid extraction and real-time RT- PCR are recommended.
38 < Ct ≤ 40	Any value		
Undetermined	38 < Ct ≤ 40	Invalid result	Repeat the procedures of nucleic acid extraction and real-time RT- PCR are recommended.

Undetermined – No amplification

LIMITATION

- The KimForest SARS-CoV-2 Detection Kit v1 is for prescription use, in vitro diagnostic use, and for use under FDA Emergency Use Authorization only. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This kit is only used for the qualitative detection of SARS-CoV-2 RNA.
- Do not rely solely on the results of this kit for a diagnosis. For a final diagnosis, the results of this kit should be considered in conjunction with the patient's symptoms, physical signs, medical history, other laboratory examinations and reactions to the treatments.
- The primers & probes have been designed to detect a highly conservative region of the RdRp gene of the virus. However, due to the high mutation rates of the RNA viruses, low possibility of mutation within the conservative regions still exists, which may lead to false negative results with this kit.
- The binding sites for the RdRp forward and reverse primers are 95% and 92% homologous, respectively to SARS coronavirus; however, no homology of the RdRp probe and SARS coronavirus was identified. Since signal from the TaqMan probe is not possible without probe binding, cross-reactivity with SARS coronavirus is not expected; however, amplification and therefore, interference with detection of SARS-CoV-2 could still occur.
- *In silico* cross-reactivity analysis of the RdRp reverse primer showed high homology with MERS-CoV; however, no

homology to the RdRp probe was exhibited and therefore, while cross reactivity is not expected with MERS-CoV it has not been definitively ruled out with wet testing.

- Based on the *in silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV may cross-react with the RdRp primer set of the KimForest SARS-CoV-2 Detection Kit v1. SARS-CoV is not known to be currently circulating in the human population, therefore it is highly unlikely to be present in patient specimens.
- Improper sampling, transportation, storage and handling may cause errors in the results.
- Validation of this kit was completed using only nasopharyngeal and oropharyngeal swabs; however, other respiratory tract specimens including anterior nasal/mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates and BALs have not been validated but are considered acceptable specimen types for use with this kit.
- Validation of this kit was only completed on the Applied Biosystems StepOnePlus Real-Time PCR System with software v2.3.
- This kit was validated for use with the Qiagen QIAamp Viral RNA Mini Kit.

CONDITIONS of AUTHORIZATION for the LABORATORY

The KimForest SARS-CoV-2 Detection Kit v1 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories using the KimForest SARS-CoV-2 Detection Kit v1, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the KimForest SARS-CoV-2 Detection Kit v1 will include with result reports of the test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
B. Authorized laboratories using the KimForest SARS-CoV-2 Detection Kit v1 will perform the test as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the KimForest SARS-CoV-2 Detection Kit v1 are not permitted.
C. Authorized laboratories that receive the KimForest SARS-CoV-2 Detection Kit v1 will notify the relevant public health authorities of their intent to run the test prior to initiating testing.
D. Authorized laboratories using the KimForest SARS-CoV-2 Detection Kit v1 will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
E. Authorized laboratories will collect information on the performance of the KimForest SARS-CoV-2 Detection Kit v1 and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and KimForest Enterprise Co., Ltd (email: danchen@geneonlink.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

F. All laboratory personnel using the KimForest SARS-CoV-2 Detection Kit v1 must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the KimForest SARS-CoV-2 Detection Kit v1 in accordance with the authorized labeling.

G. KimForest Enterprise Co., Ltd., authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1]For ease of reference, this refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

PERFROMANCE CHARACTERISTICS

Limit of Detection

The LoD study established the lowest SARS-CoV-2 RNA concentration (copies/mL) that consistently yielded a ≥ 95% positivity rate with the KimForest SARS-CoV-2 Detection Kit v1.

A preliminary LoD for the SARS-CoV-2 specific target (RdRp) was determined using heat inactivated SARS-CoV-2 virus obtained from BEI Resources (Cat # NR-52286; Lot No.: 70034991). The Certificate of Analysis (CoA) of NR-52286 indicated a concentration of 3.75 x 10^5 genome equivalents/µL. Clinical, pooled nasopharyngeal swab matrices were screened negative using an EUA authorized RT-PCR assay and spiked with viral genomic RNA at various concentrations (1000, 500, 300, 250, 200, and 150 copies/mL). Spiked samples at each concentration were tested in triplicate on the Applied Biosystems StepOnePlus Real-Time PCR System (**Table 3**). The preliminary LoD using nasopharyngeal swab matrix was determined to be 200 copies/mL.

Table 3. Preliminary LoD Study Data Generated with Spiked Samples at Various Concentrations Tested in Triplicate

Concentration (genome copies/mL)	Mean Ct Values						Average Ct of RdRp	Detection Rate
	Replicate # 1		Replicate #2		Replicate #3			
	RdRp	RNase P	RdRp	RNase P	RdRp	RNase P		
1,000	30.525	26.707	30.519	26.683	30.172	26.619	30.405	3/3
500	30.821	26.458	31.492	26.493	31.239	26.435	31.184	3/3
300	31.032	25.686	31.622	25.661	31.711	25.634	31.455	3/3
250	31.465	26.476	31.516	26.336	31.707	26.550	31.563	3/3
200	32.369	26.911	32.056	26.959	31.634	26.906	32.020	3/3
150	31.874	25.936	UND	25.748	31.794	26.175	N/A	2/3

The LoD of the KimForest SARS-CoV-2 Detection Kit v1 was confirmed using 20 individual extraction replicates consisting of spiked nasopharyngeal swab samples at 200 copies/mL. Samples were extracted with the QIAamp Viral RNA Mini Kit and tested on the Applied Biosystems StepOnePlus Real-Time PCR System. The lowest target level at which more than 95% of 20 replicates for nasopharyngeal swab specimens produced positive results was 200 copies/mL (**Table 4**).

Table 4. LoD Confirmation Study Results

Spike-in viral genome copies (200 copies/mL)					
Samples	RdRp	RNase P	Samples	RdRp	RNase P
Sample 1	31.268	27.785	Sample 11	30.770	27.971

Sample 2	32.990	28.437	Sample 12	31.205	27.661
Sample 3	30.985	27.977	Sample 13	31.429	27.698
Sample 4	30.927	27.758	Sample 14	31.449	27.966
Sample 5	31.094	27.774	Sample 15	30.676	27.597
Sample 6	31.223	27.984	Sample 16	31.017	27.705
Sample 7	31.086	27.957	Sample 17	31.252	27.757
Sample 8	31.080	27.799	Sample 18	30.935	27.618
Sample 9	30.792	27.792	Sample 19	31.494	27.793
Sample 10	31.893	27.752	Sample 20	31.196	27.789
Agreement (%)			**20/20 (100%)**		

Inclusivity

To demonstrate the predicted inclusivity of the KimForest SARS-CoV-2 Detection Kit v1, *in silico* analysis was performed to verify the RdRp primer and probe sequence homology to publicly available SARS-CoV-2 sequences in the GISAID database. As of August 11, 2020, all complete SARS-CoV-2 genomes defined by GISAID as >29,000 bp from human hosts of which the N proportion of the genome was < 5% were enrolled for inclusivity analysis. A total of 74,407 isolates met enrollment criteria and were evaluated for inclusivity. The 74,407 viral genomes were aligned against RdRp primers and probe used in KimForest SARS-CoV-2 Detection Kit v1. The number of primer/probe mismatches to the deposited viral genomes were analyzed and summarized in the table below (**Table 5**).

According to inclusivity study results, without considering N base within the primer/probe aligned region, all sequences showed less than or equal to 3 base mismatches. For the SARS RdRp_R inclusivity study, SARS RdRp_R was designed with 26 nucleotides in length, and 73 sequences showed 3 mismatches against the SARS RdRp_R sequence. For the SARS RdRp_P inclusivity study, SARS RdRp_P was designed with 25 nucleotides in length, and only one sequence showed 3 mismatches against the SARS RdRp_P sequence. Therefore, it is predicted that the KimForest SARS-CoV-2 Detection Kit v1 should detect all deposited SARS-CoV-2 sequences within GISAID.

Table 5. *In silico* Analysis for the Detection of SARS-CoV-2 Sequences

	1 base mismatch	2 bases mismatch	3 bases mismatch	N≥1 base (within primer/probe region)	Perfect match	Total isolates
SARS RdRp_F	296	8	0	59	74,045	74,407
SARS RdRp_R	74,251	43	73	38	2	74,407
SARS RdRp_P	45	2	1	20	74,339	74,407

Analytical Specificity (Cross-Reactivity)

Potential cross-reactivity against normal and pathogenic organisms associated with the respiratory tract was assessed through *in silico* analysis of each RdRp primer and probe sequence. The RdRp primer and probe set was mapped against sequences downloaded from NCBI GenBank, Candida Genome database, and GISAID. No potential unintended cross-reactivity to other pathogens except for SARS coronavirus is expected (See **Table 6**). The binding sites for the RdRp forward and reverse primers are 95% and 92% homologous, respectively to SARS coronavirus; however, no homology of the RdRp probe and SARS was identified. Since the TaqMan probe signal is not possible without probe binding, cross-reactivity with SARS coronavirus is not expected.

Table 6. Cross-Reactivity Results Summary

Other high priority pathogens from the same genetic family	Accession ID	Percent Homology		
		SARS-CoV-2_RdRp forward primer	SARS-CoV-2_RdRp reverse primer	SARS-CoV-2_RdRp probe
Human coronavirus 229E	MF542265	0%	0%	0%
Human coronavirus 229E	MN369046	0%	0%	0%
Human coronavirus 229E	MN306046	0%	0%	0%
Human coronavirus 229E	KY996417	0%	0%	0%
Human coronavirus OC43	MK303620	0%	0%	0%
Human coronavirus OC43	MN310478	0%	0%	0%
Human coronavirus OC43	MG197719	0%	0%	0%
Human coronavirus OC43	MG197713	0%	0%	0%
Human coronavirus OC43	NC_006213	0%	0%	0%
Human coronavirus HKU1	MK167038	0%	0%	0%
Human coronavirus HKU1	MH940245	0%	0%	0%
Human coronavirus HKU1	KF686341	0%	0%	0%
Human coronavirus HKU1	NC_006577	0%	0%	0%
Human coronavirus NL63	MK334047	0%	0%	0%
Human coronavirus NL63	MN306040	0%	0%	0%
Human coronavirus NL63	NC_005831	0%	0%	0%
Human coronavirus NL63	JQ765567	0%	0%	0%
Human coronavirus NL63	JQ765573	0%	0%	0%
Human coronavirus NL63	JQ765564	0%	0%	0%
SARS Coronavirus	NC_004718.3	95% (21/22 bases)	92% (24/26 bases)	0%
SARS Coronavirus TW1	AY291451.1	95% (21/22 bases)	92% (24/26 bases)	0%
SARS Coronavirus Sin2677	AY283795.1	95% (21/22 bases)	92% (24/26 bases)	0%
MERS	MN120514.1	0%	80.8% (21/26 bases)	0%
MERS	MH013216.1	0%	80.8% (21/26 bases)	0%
MERS	KP719932.1	0%	80.8% (21/26 bases)	0%
Human adenovirus 71 strain	KF268207	0%	0%	0%
Human adenovirus C	NC_001405	0%	0%	0%
Human metapneumovirus	NC_039199	0%	0%	0%
Human parainfluenza virus 1	NC_003461	0%	0%	0%
Human parainfluenza virus 2	KM190939	0%	0%	0%
Human parainfluenza virus 3	NC_001796	0%	0%	0%
Human parainfluenza virus 4	NC_021928	0%	0%	0%
Influenza A virus[#]		0%	0%	0%
Influenza B virus[$]		0%	0%	0%
Human enterovirus 68	JX070222	0%	0%	0%
Enterovirus D68	KU509997	0%	0%	0%
Respiratory syncytial virus	KM360090	0%	0%	0%
Human respiratory syncytial virus A	MF614947	0%	0%	0%
Human respiratory syncytial virus B	MG642045	0%	0%	0%
Human rhinovirus 2	X02316	0%	0%	0%
Human rhinovirus 14	NC_001490	0%	0%	0%
Chlamydia phage	NC_002180	0%	0%	0%
Haemophilus influenzae	NC_017451	0%	0%	0%
Legionella pneumophila	NC_021350	0%	0%	0%

Legionella pneumophila	NC_016811	0%	0%	0%
Mycobacterium tuberculosis	NC_017524	0%	0%	0%
Mycobacterium tuberculosis	NC_022350	0%	0%	0%
Mycobacterium tuberculosis	NC_022350	0%	0%	0%
Streptococcus pneumoniae	NC_011900	0%	0%	0%
Streptococcus pneumoniae	NC_018594	0%	0%	0%
Streptococcus pneumoniae	NC_014494	0%	0%	0%
Streptococcus pyogenes	NC_017596	0%	0%	68% (17/25 bases)
Streptococcus pyogenes	NC_011375	0%	0%	0%
Bordetella pertussis	NC_017223	0%	0%	0%
Mycoplasma pneumoniae	NC_000912	0%	0%	0%
Mycoplasma pneumoniae	NC_016807	0%	0%	0%
Pneumocystis jirovecii RU7	LFWA01000000	0%	0%	0%
Candida albicans[&]		0%	0%	0%
Pseudomonas aeruginosa	NC_008463	0%	0%	0%
Pseudomonas aeruginosa	NC_009656	0%	0%	0%
Staphylococcus epidermidis	NC_004461	0%	0%	0%
Streptococcus salivarius	NC_017595	0%	0%	0%
Streptococcus salivarius	NZ_CP015283	0%	0%	0%

[#]Influenza A virus genome is combined from segment 1 to 8 (NC_002023, NC_002021, NC_002022, NC_002017, NC_002019, NC_002018, NC_002016, NC_002020).
[$]Influenza B virus genome is combined from segment 1 to 8 (NC_002204, NC_002205, NC_002206, NC_002207, NC_002208, NC_002209, NC_002210, NC_002211).
[&]*Candida albicans* genome is taken from Candida Genome Database

Clinical Evaluation

Performance of the KimForest SARS-CoV-2 Detection Kit v1 was evaluated using a retrospective cohort of 30 positive and 30 negative samples that were previously tested with an FDA authorized molecular SARS-CoV-2 assay. A total of 30 positives (20 nasopharyngeal swabs and 10 oropharyngeal swabs) and 30 negatives (20 nasopharyngeal swabs, 9 oropharyngeal swabs, and 1 sample where the swab type was not indicated) were tested to validate the clinical performance of the KimForest SARS-CoV-2 Detection Kit v1 performed on the Applied Biosystems StepOnePlus Real-Time PCR System.

The KimForest SARS-CoV-2 PCR Detection Kit v1 correctly detected 30/30 positives and 30/30 negative clinical specimens. The positive and negative percent agreements between the KimForest SARS-CoV-2 Detection Kit v1 and the authorized EUA molecular comparator assay are shown below in **Table 7**.

Table 7. Summary of the Clinical Evaluation of the KimForest SARS-CoV-2 Detection Kit v1 Compared to Another EUA Authorized RT-PCR Assay

Nasopharyngeal and Oropharyngeal Swabs Combined Performance		**EUA Authorized Comparator Test**		
		Positive	**Negative**	**Total**
KimForest SARS-CoV-2 Detection Kit v1	**Positive**	30	0	30
	Negative	0	30*	30
	Total	30	30	60
Positive Percent Agreement (PPA)		30/30; 100% (95% CI 88.65-100.00%)[1]		
Negative Percent Agreement (NPA)		30/30; 100% (95% CI 88.65-100.00%)[1]		

[1]Two-sided 95% score confidence intervals
*One negative sample had an unknown specimen type

REFERENCES

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2. 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes announced by Centers for Disease Control and Prevention (CDC), US
3. CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel

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MARKING SYMBOLS

Symbols	Definition
CE	Product conforms with the essential requirements of applicable EC directives
REF	Catalog number
Cont.	Kit contents
	Caution
	Manufacturer
	Test number
EC REP	Authorized representative in the European community
LOT	Batch code
	Expired date
	Store at -15~-25℃
	Consult instructions for use