CARBON
TECHNOLOGIES

Stark Sars-CoV-2
Molecular Diagnostic Kit

IVD
CE

Contents

Description

In the waning days of 2019, a novel strain of Coronavirus precipitated a global epidemic of pneumonia. As fatalities from Covid-19 surpassed 1000, the World Health Organization (WHO) christened the disease caused by SARS-CoV-2 as COVID-19. Initially dubbed 2019-nCoV (novel Coronavirus), the virus was later rebranded as SARS-CoV-2 by the International Committee on Taxonomy of Viruses. This marked the first instance of such a virus inflicting widespread human infection. Early estimates pegged the mortality rate of SARS-CoV-2 at 2 to 3%. Typical symptoms among infected individuals include fever, dry cough, and respiratory distress characterized by shortness of breath or severe breathing difficulties, alongside sore throat and runny nose. While the initial cases of COVID-19 were traced back to a seafood market in the Wuhan region of China, initial suspicions of zoonotic transmission swiftly gave way to acknowledgment of human-to-human spread by the World Health Organization.

Intended Use

The Stark Sars-CoV-2 Molecular Diagnostic Kit employs a reverse transcription-PCR assay tailored to specifically detect the virus, with its detection facilitated by Real-Time PCR. This Kit is designed for the qualitative detection of nucleic acid from SARS-CoV-2 in samples obtained from nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, nasal washes, and nasal aspirations from individuals suspected of COVID-19 by their healthcare provider. Testing is restricted to laboratories certified under the Clinical Laboratory Improvement Amendment. In adherence to regulations set forth by the Ministry of Health, the sale of COVID-19 diagnostic Kits is permitted solely to licensed laboratories. This Kit is intended for emergency use and for In Vitro Diagnostic (IVD) purposes. Additionally, the test serves to identify the genome of SARS-CoV-2, which is discernible in samples from patients with acute viral infections. A positive result indicates the presence of the SARS-CoV-2 genome; however, accurate diagnosis necessitates consideration of the patient's clinical history and other medical conditions. It is imperative to note that positive results do not definitively rule out bacterial infection or co-infection with other viruses. The detected agent may not be the sole causative agent of the disease. Laboratories are obligated to report all positive cases to the relevant public health authorities. Conversely, negative results do not conclusively exclude SARS-CoV-2 infection and should not be the sole basis for patient management decisions. Negative results should be interpreted in conjunction with clinical observations, patient history, and epidemiological data.

Kit Content

Components	100 Preps (REF: ST242004)
Q-ROMAX, 4X	500µl
Prol Mix	400µl
RTase, Recombinant Reverse Transcriptase, RNase H-(200 U/µl)	100µl
Positive Control	150µl
Negative control	150µl

Storage

All components of the Stark MTB Molecular Diagnostic Kit are pre-prepared and ready for immediate use upon arrival. Upon receipt, it is recommended to store all reagents at temperatures ranging from -15°C to -30°C. These conditions ensure stability and maintain the integrity of the components until the expiration date indicated on the label.

Guarantee and Warranty

CARBON Technologies LLC stands behind the efficacy of all manufactured Kits and reagents. If you need assistance in choosing the right Kits for your needs, our technical support team is available to provide guidance. Should the products not meet your expectations due to reasons other than misuse, please do not hesitate to contact our

technical support team. In the rare event of issues arising from the manufacturing process, CARBON Technologies LLC will promptly replace the Kit.

Warning and Precautions

- Material Safety Data Sheets (MSDS) for all products and reagents are available online at www.carbontechnologiesco.com.
- Please adhere to laboratory safety protocols.
- Prior to use, thoroughly review the guidelines provided.
- Note that all patient samples and positive controls carry the potential for infectiousness.
- Refrain from eating, drinking, smoking, chewing gum, applying cosmetics, or taking medication while handling hazardous materials and human samples in laboratories. Treat all patient samples and positive controls as potentially infectious.
- Before commencing work, familiarize yourself with all safety guidelines related to handling COVID-19 samples, accessible at https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- The Stark Sars-CoV-2 Molecular Diagnostic Kit is intended for use by qualified and trained clinical laboratory personnel who have received specific instruction and training in real-time PCR and in vitro diagnostic procedures. All steps of the procedure, including sampling, storage, shipping, and laboratory tests, must adhere to biosafety and laboratory information management system (LIMS) protocols.
- This test requires a separate and dedicated workstation within the laboratory:
    - Location 1: Preparation area - for assembling test components.
    - Location 2: Sample processing area - for sample isolation and handling.
    - Location 3: Amplification area - for conducting Real-Time PCR tests.
- Clinical laboratories must be equipped with instruments and personnel in accordance with guidelines from the Ministry of Health.
- The contents provided in the Kit are specific to COVID-19 testing. Altering or substituting any Kit contents may compromise product performance.
- Before commencing tests, ensure that each component is thawed, vortexed, and briefly centrifuged. Avoid subjecting components to repeated freeze-thaw cycles.
- All pipette tips and microtubes must be sterile and free from DNase and RNase contamination. Use filter pipette tips to prevent contamination, and change tips after adding any substances or samples.
- Dispose of hazardous and biological waste in compliance with local and national regulations to prevent environmental contamination. Use decontaminants such as 10% sodium hypochlorite, 70% ethanol, and Surface sanitizing solution for nucleic acid contaminations. Avoid exposing PCR-COVID 19 combinations to sunlight.
- Positive results must be promptly reported to health authorities.

Quality Control

The Stark Sars-CoV-2 Molecular Diagnostic Kit undergoes rigorous testing in accordance with standards set by the Clinical and Laboratory Standards Institute and the World Health Organization (WHO). These tests are conducted on a lot-to-lot basis to uphold consistent product quality. For detailed information regarding the results of these tests, please visit www.carbontechnologiesco.com and enter the labeled REF and LOT number in the "Certificate of Analysis" section.

Materials Required (but Not Provided)

- Nylon or Dacron swab with an aluminum or plastic shaft.
- DNase-RNase-free microtubes for sampling (1.5ml).

- PCR microtube 0.1ml or 0.2ml strip.
- Various models of pipettes and pipette tips (10µl, 100µl, and 1000µl of filter pipette tips).
- Surface sanitizing solution.
- Disposable Powder-Free gloves and surgical gown.
- Different types of Real-Time PCR Instruments (with green, yellow, and orange channels).
- Centrifuge (capable of reaching 13000 rpm).
- Microcentrifuge.
- Vortex.
- Cool box

Real-Time PCR Instruments

This Kit is compatible with the following instruments:

- Rotor-Gene Q, 5plex
- Corbett Rotor-Gene 3000 & 6000
- Mic qPCR Cycler
- ABI StepOne & StepOnePlus
- Biorad CFX96 Real-Time PCR
- Roche LightCycler® 96 Real-Time PCR System
- Anatolia Montania 484 Real-Time PCR Instrument

Applications

The Stark Sars-CoV-2 Molecular Diagnostic Kit is a Real-Time reverse transcription-polymerase chain reaction (rRT-PCR) test specifically designed for the detection of RNA from SARS-CoV-2 in respiratory specimens obtained from patients suspected of COVID-19 by their healthcare provider. This Kit enables qualitative detection of the RdRp and N genes of SARS-CoV-2 RNA.

With a simple centrifugation and lysis step, the sample mixture can be directly added to the 2019-nCoV-PCR master mix (Q-ROMAX+ RTase+ ProI) for rRT-PCR amplification. An internal control targeting the RNase P gene is incorporated to monitor the entire process, from sample collection to rRT-PCR, ensuring the accuracy of results and minimizing the risk of false-negative outcomes. The Limit of Detection (LoD) of the Kit is 100 copies/ml, providing reliable sensitivity for the detection of SARS-CoV-2 RNA in clinical specimens.

Recommended Starting Material

- Sample Collection

The Stark Sars-CoV-2 Molecular Diagnostic Kit is designed for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory specimens. It is crucial to ensure proper collection procedures to minimize the risk of contamination during collection, storage, and transportation. Specimens should be handled with caution and considered potentially infectious, adhering to relevant regulations and biosafety guidelines. Synthetic-tipped swabs, such as nylon or Dacron, with aluminum or plastic shafts are recommended for collection. Cotton swabs with wooden shafts should be avoided. After sampling, swabs should be promptly placed in a suitable virus transport medium.

- Storage and Delivery of Specimens

Specimens should ideally be tested within 24 hours if stored at 4°C. For samples that cannot be tested within this timeframe, storage at -70°C or below is recommended. Alternatively, specimens can be stored at -20°C for up to ten days. Nucleic acid extracted from specimens can be stored at -20±5°C for up to 15 days. It is essential to avoid multiple freeze-thaw cycles to maintain sample integrity.

- Specimen Isolation

For viral nucleic acid isolation, use a Kit which is approved by the Ministry of Health should be used.

- Pathogenicity

Coronaviruses can cause a spectrum of illnesses ranging from mild cold-like symptoms to severe respiratory diseases. Symptoms commonly associated with Coronavirus infections include fever, cough, shortness of breath, and respiratory difficulties. Some patients may experience persistent coughing without an apparent cause. In contrast to SARS-CoV, MERS-CoV primarily affects the respiratory system and can lead to complications such as kidney and liver damage. Severe cases of Coronavirus infections may present with additional symptoms such as diarrhea, acute respiratory distress syndrome (ARDS), coagulopathy, and renal failure, necessitating interventions such as hemodialysis.

COVID-19, caused by the novel SARS-CoV-2 virus, typically manifests symptoms within a few days of exposure. However, symptom onset may vary among individuals. Fever is a common symptom, observed in 43.8% of cases upon hospital admission and in 88.7% of hospitalized cases. Dry cough is prevalent in 67.8% of cases, accompanied by respiratory distress, fatigue, and myalgia in 11 to 14% of patients. Diarrhea is reported in 3.8% of cases. The average incubation period for COVID-19 is approximately four to five days. Ground-glass opacity, a characteristic finding on chest CT scans, is observed in 56.4% of cases during the incubation period. Despite this, 17.9% of patients with mild symptoms and 2.9% with severe symptoms may not exhibit abnormalities on radiological imaging. Lymphopenia, characterized by a decreased number of lymphocytes in the blood, is reported in 83.2% of patients upon hospital admission. While some individuals may experience mild or asymptomatic infections, COVID-19 can lead to severe complications such as pneumonia or acute respiratory distress syndrome, particularly in patients with underlying medical conditions, posing a significant risk of mortality.

- Transmission of Coronavirus

The SARS-CoV-2 virus can spread through airborne particles produced by coughing or sneezing, similar to the flu. Close contact with infected individuals, particularly in indoor settings, poses a higher risk of transmission. While outdoor environments generally carry a lower risk, prolonged indoor exposure to infected individuals, such as hospitalized COVID patients, can facilitate human-to-human transmission. The exact mode of transmission, whether from animal-to-human or through contaminated surfaces, is still under investigation.

- Workstation Preparation

Before commencing work, ensure all work surfaces, pipettes, centrifuges, and other equipment are thoroughly cleaned and sanitized to minimize the risk of nucleic acid contamination. Sanitizers such as 70% Ethanol or 10% Sodium Hypochlorite should be used for effective disinfection.

Protocol

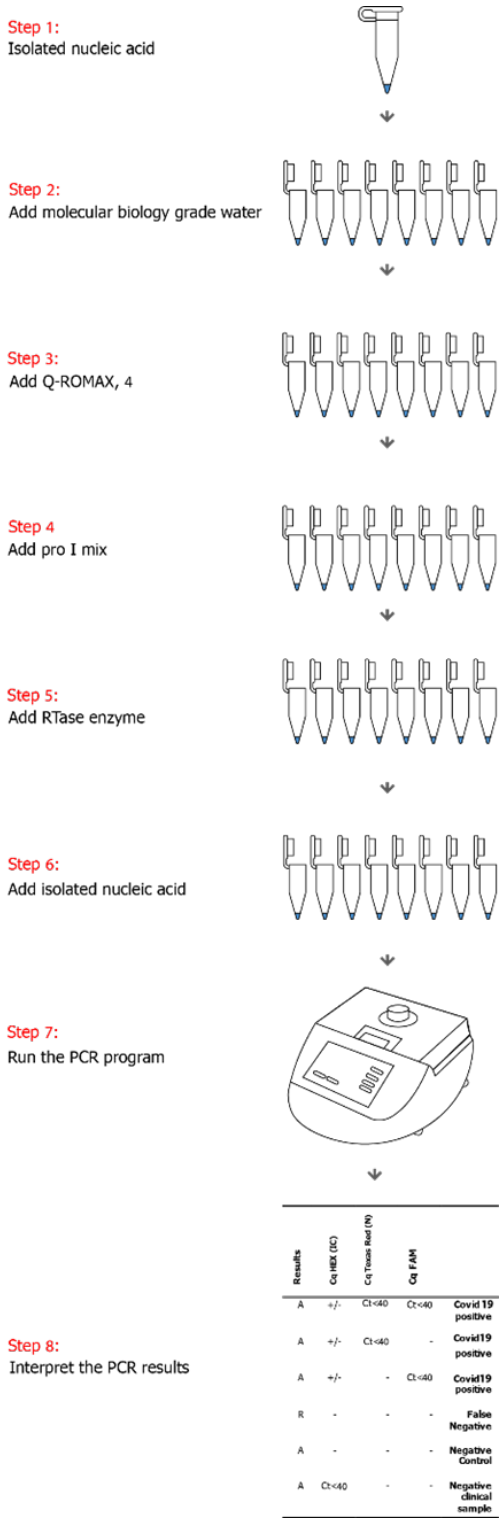

Figure 1: Preparation of reagents, the addition of isolated DNA, PCR run, and interpretation of results.

Process

- Remove each component from the diagnostic Kit and place them on the bench at room temperature.
- Allow the reagents to equilibrate to room temperature.
- After equilibration, briefly vortex each component to ensure homogeneity for later use.
- Ensure that the isolated sample volume for this test is 10µl.
- Refer to Table 1 to prepare the reaction components (Master Mix) according to the specified quantities.
- Perform Real-Time PCR according to the instructions provided in Table 2.

Table1: Regent's preparation per one single reaction.

Components	Volume
Q-ROMAX	5µl
RTase, Recombinant Reverse Transcriptase, RNase H-(200 U/µl)	1µl
Pro I Mix	4µl
Isolated RNA	10µl

Table 2: PCR program for one-step Multiple Real Time-RT-PCR.

Step	Time	Temperature	Number of cycles
cDNA synthesis	20min	50ºC	1
Polymerase enzyme activation	1min	95ºC	1
Denaturation	10s	95ºC	45 cycles
Annealing and extension of nucleic acid and measurement of fluorescence in green, yellow, and orange channels	40s	60ºC	

Interpretation of Clinical Results

- Perform data analysis for each gene separately using a manual threshold.
- Ensure that the threshold for each sample is within the exponential phase of the fluorescence curves and above any background signal.
- Utilize FAM Fluorophore (green) for the RdRp gene, Texas Red Fluorophore (orange) for the N gene, and HEX Fluorophore (yellow) for the RNase P gene (internal control).
- Employ a negative control as contamination control, ensuring that the fluorescence curve magnitude does not cross the threshold. Ct values less than 35 (Ct<35) indicate possible contamination. Strong signals above 35 in the NTC may indicate PCR artifacts, and in such cases, the shape of the curve should be considered (the S-shaped curve is typical for a positive result).
- Ensure that the internal control or RNase P gene is positive for all clinical specimens with a Ct value of 35 or less, indicating sufficient nucleic acid from the human RNase gene and acceptable sample quality.
- If the internal control curve or RNase P gene Ct value is greater than 40 or absent, it indicates low sample concentration or inhibitors in the reaction. In such cases, the isolated sample is recommended to be diluted by at least half. If the test result is not acceptable upon retest, obtain a new sample from the patient, and repeat the test.
- A positive clinical specimen should have a Ct value of ≤40 for genes or should exhibit positivity for two genes.

- If the expected positive reaction is not achieved (typical S-shaped curve), the performed test is deemed unacceptable, and the test must be repeated while accurately following the Kit instructions.
- Identify the reason for the failure of the positive control, take corrective action, and document the results of the corrective action.

Table 3: Control conditions for a valid PCR Run

RdRp (FAM)	N (Texas Red)	IC (HEX)	Results
+	+	Not considered	Positive control*
+	-		
-	+		
-	-	+	Negative control
-	-	-	Invalid and not accepted

Result of (-): Ct value >40 or Undetermined, Result of (+): Ct value ≤ 40*

Test Limitations

- A false-negative result may occur due to low titration of the virus in the patient sample, improper transportation, and poor quality of sample isolation.
- The genetic diversity of the coronavirus genome can lead to poor primer/probe binding to the target sequence, resulting in false-negative results, despite attempts to design primers or probes for conserved viral genome regions.
- All controls must be verified before result interpretation. Invalid controls render the patient's results uninterpretable. The diagnostic limit of this Kit is Ct≤40, and users must review the fluorescence curve before final interpretation, ensuring all positive curves exhibit an amplification peak.
- Failure to adhere to proper storage conditions for the Kit can lead to false-negative results.
- Proper handling of this Kit requires experienced and trained personnel. Errors by personnel may lead to invalid results.
- Results obtained from this diagnostic Kit are only acceptable when combined with clinical evidence for diagnosing SARS-CoV-2. Definitive diagnosis and treatment of patients should be based on a combination of this test with other test results, medical records, and response to treatment.

Performance Evaluation

- Standard Sample Preparation

RNA was isolated from a sample contaminated with SARS-CoV-2 (200,000 copies per ml). Serial dilutions of 20, 200, 2,000, and 20,000 copies per ml were prepared from the same sample. Using the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech Inc.), three replicates were tested for each dilution using Real-Time PCR (Rotor-Gene Q-Qiagen). The final concentration of positive samples for N and RdRp is provided in the table below.

Table 4: Average Ct results of Sansure Biotech Novel Coronavirus (nCOV-2019) Nucleic Acid Diagnostic Kit detecting N gene and RdRp gene.

Target genes	concentration (copies/mL)	Average of Cts three repeats	Average of Cts three repeats for a limit of detection in Kit (200copies/mL)
N	200000	22.9587	36.17

	20000	26.61745	
	2000	30.65462	
	200	36.17117	
	20	38.99604	
RdRp	200000	24.41095	37.21
	20000	27.98925	
	2000	31.68987	
	200	37.21188	
	20	38.9026	

The same dilutions were evaluated by Stark Sars-CoV-2 Molecular Diagnostic Kit, and the results are summarized in Table 5 below:

Table 5: Average Ct results of Stark Sars-CoV-2 Molecular Diagnostic Kit.

Target genes	Concentration (copies/mL)	Average of Cts three repeats	Average of Cts three repeats for a limit of detection in Kit (200copies/mL)
N	200000	24.00423	35.32749
	20000	27.65744	
	2000	31.82054	
	200	35.32749	
	20	Undetermined	
RdRp	200000	22.97669	34.46268
	20000	26.91568	
	2000	30.91477	
	200	34.46268	
	20	Undetermined	

- Limit of Detection (LoD) - Analytical Sensitivity

LoD studies were conducted to ascertain the lowest detectable concentration of SARS-CoV-2 RNA, where approximately 95% of all true positive replicates test positive. These studies employed limiting dilution techniques using characterized samples.

LoD with Clinical Specimen

The LoD of the Stark Sars-CoV-2 Molecular Diagnostic Kit was estimated by testing standardized dilutions of the positive specimen. These dilutions were serially diluted to 200 copies/mL, 100 copies/mL, and 50 copies/mL (n=3 each) using a negative specimen matrix (a negative oropharyngeal swab specimen). The lowest concentration at which all three replicates tested positive was considered the tentative LoD for each test. The LoD of each test was then confirmed by testing 20 replicates with concentrations at the tentative detection limit. The final LoD of each test was determined to be the lowest concentration resulting in positive detection in 19 out of 20 replicates.

The LoD of the Stark Sars-CoV-2 Molecular Diagnostic Kit was established using a nucleic acid isolation Kit, revealing an LoD of 100 copies/mL. LoD Detection Results of 2019-nCoV using a nucleic acid isolation Kit:

Table 6: Analytical sensitivity and Limit of Detection of Stark Sars-CoV-2 Molecular Diagnostic Kit

No	Concentration (Copies/mL)					
	RdRp gene			N gene		
	200	100	50	200	100	50
1	37.33546	36.56248	Undetermined	36.7778	36.01872	Undetermined
2	35.75644	37.2996	Undetermined	34.97221	35.72504	Undetermined
3	36.15275	36.45737	40.01379	35.50759	35.77135	39.49453
4	36.12224	37.92434	39.78859	35.45836	37.33982	39.17679
5	35.43289	36.67382	Undetermined	34.66985	35.91962	Undetermined
6	36.18688	36.99414	Undetermined	35.50755	36.35858	Undetermined
7	35.79122	37.54021	Undetermined	35.2	37.05488	Undetermined
8	35.92614	36.86021	Undetermined	35.28196	36.22875	Undetermined
9	35.81124	37.07031	Undetermined	35.20401	36.47231	Undetermined
10	35.73511	37.16536	Undetermined	34.94667	36.53217	Undetermined
11	35.88995	36.75371	40.21012	35.23834	36.22711	39.60618
12	36.50063	37.4638	Undetermined	35.73087	36.7232	Undetermined
13	36.46613	37.22571	Undetermined	35.92348	36.61359	Undetermined
14	35.73911	36.62986	Undetermined	35.17391	36.03484	Undetermined
15	36.45601	37.04602	Undetermined	36.02764	36.44575	Undetermined
16	36.0982	37.04296	Undetermined	35.44752	36.35696	Undetermined
17	36.52337	37.11725	40.11924	35.36263	36.82512	39.93521
18	35.43264	37.159251	Undetermined	35.62663	37.062543	40.16625
19	35.735532	36.271552	40.03261	35.72521	36.24283	39.96241
20	36.639926	37.623919	39.76625	35.76523	36.762191	40.02371
Call rate	100%	100%	30%	100%	100%	35%

- Inclusivity (Analytical Sensitivity)

The inclusivity of the primer/probe set utilized in the Stark Sars-CoV-2 Molecular Diagnostic Kit was assessed in silico, leveraging SARS-CoV-2 sequences from the NCBI database (3612 sequences) accessed on August 2, 2020. The alignment analysis of primer/probe sets targeting the RdRp gene and N gene sequences demonstrated 100% inclusivity for SARS-CoV-2 sequences identified from patient samples.

Table 7: Representative results of In Silico Analysis for Coronavirus (2019-nCOV) primers/probe against the reported 2019-nCOV sequences in NCBI Site.

Strain	Target	Accession	% Homology Test Forward primer%	% Homology Test Reverse primer%	% Homology Test Probe%
SARS-CoV-2/Felis catus/USA/TAMU-078/2020	N gene	MW263337.1	100	100	100

SARS-CoV-2/Canis familiaris/USA/TAMU-077/2020	N gene	MW263336.1	100	100	100
SARS coronavirus isolate Xiao Tang Shang Hospital polyprotein 1ab-like gene	N gene	AY465926.1	100	100	100
SARS coronavirus Tor2 isolate Tor2/FP1-10912	N gene	JX163923.1	100	100	100
SARS-CoV-2/human/TWN/CGMH-CGU-36/2020	N gene	MW356672.1	100	100	100
SARS-CoV-2/human/ECU/Z&Z_SARS_4/2020	N gene	MW294011.1	100	100	100
BetaCoV/Wuhan/WH-01/2019	N gene	CNA0007332	100	100	100
SARS coronavirus isolate Guangdong/20SF012/2020	N gene	EPI_ISL_403932	100	100	100
SARS coronavirus isolate /South Korea/KCDC03/2020	N gene	EPI_ISL_407193	100	100	100
SARS coronavirus isolate /France/IDF0372/2020	N gene	EPI_ISL_406596	100	100	100
SARS coronavirus isolate /Finland/1/2020	N gene	EPI_ISL_407079	100	100	100
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/TX-DSHS-1535/2020	N gene	MW349166.1	100	100	100
SARS-CoV-2/human/TWN/CGMH-CGU-31/2020	N gene	MW356784.1	100	100	100
SARS coronavirus isolate /Japan/AI/I-004/2020	N gene	EPI_ISL_407084	100	100	100
SARS coronavirus isolate /England/01/2020	N gene	EPI_ISL_407071	100	100	100
SARS-CoV-2/human/TWN/CGMH-CGU-36/2020	N gene	MW356672.1	100	100	100
SARS coronavirus isolate /Singapore/1/2020	N gene	EPI_ISL_406973	100	100	100
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/MN-MDH-2049/2020	N gene	MW349104.1	100	100	100
SARS coronavirus isolate /Australia/VIC01/2020	N gene	EPI_ISL_406844	100	100	100

- Clinical Sensitivity

The clinical sensitivity of the Stark Sars-CoV-2 Molecular Diagnostic Kit was evaluated through wet testing of inclusivity using a nucleic acid isolation Kit. Three SARS-CoV-2 positive specimens from the Day hospital were tested, each confirmed positive by the rRT-PCR Kit.

Each specimen was diluted to concentrations corresponding to ≤3log10 LOD, ≤2log10 LOD, and ≤1log10 LOD in a negative specimen matrix (Nasal/oropharyngeal swab specimen) and tested in triplicate.

Table 8: Clinical sensitivity results of Stark Sars-CoV-2 Molecular Diagnostic Kit

Concentration	Specimen	Ct N gene	Ct RdRp gene
≤3log10 LOD	Nasal/oropharyngeal swab	25.423	22.864
		25.32	22.766
		25.472	23.01
≤2log10 LOD	Nasal/oropharyngeal swab	27.767	26.156
		27.659	26.229
		27.821	26.162
≤1log10 LOD	Nasal/oropharyngeal swab	32.20	31.91477
		32.431	31.892
		32.268	31.9371

- **Cross-reactivity (Analytical Specificity)**

The Stark Sars-CoV-2 Molecular Diagnostic Kit underwent evaluation for cross-reactivity through both in silico analysis and wet testing of potentially cross-reactive pathogens or purified nucleic acid from clinical specimens. No instances of cross-reactivity were detected.

The in-silico mapping analysis of each primer/probe against various pathogens utilized the NCBI nr/nt database accessed on August 12, 2020, employing BLASTN 2.10.0+. Representative results are provided in the table below. Notably, the primer/probes targeting the RdRp and N genes may exhibit detection of bat coronaviruses based on this in silico analysis. Additionally, no cross-reactivity was observed for other listed respiratory pathogens in both in silico and wet-testing scenarios.

Table 9: The in-Silico Specificity Analysis of primer and Probe sets Coronavirus (nCOV-2019) for other Respiratory pathogens.

Pathogen (Taxonomy ID)	Strain	Target	GenBank Acc#	% Homology Test FP	% Homology Test RP	% Homology Test Probe
Human coronavirus 229E	camel/Abu Dhabi/B38	N gene	MG000870.1	43	36	40
Human coronavirus OC43	HCoV_OC43/Seattle/USA/SC9428/2018	N gene	MN630549.1	63	58	33
Human coronavirus HKU1	HKU1 SC2628	N gene	DQ437612.1	71	36	30
Human coronavirus	HCoV_NL63/Seattle/USA/SC0179/2018	N gene	DQ462758.1	56	36	43

NL63						
MERS-CoV	BtVs-BetaCoV/SC2013	N gene	MK858156.1	43	54	63
Human adenovirus D8 isolate BA_280-2008 hexon gene	Human	N gene	MK913814.1	78	60	40
Human metapneumovirus	Human	N gene	MH482736.1	47	40	40
Paramyxoviridae	MVs/Padova. ITA/24.17/1[B3]	N gene	MK513622.1	52	48	40
Orthomyxoviridae	A/ruddy turnstone/South Carolina/UGAI18-1224/2018 (H3N1)	N gene	MN938062.1	52	48	43
Parainfluenza	NCTC10665	N gene	LR134481.1	58	65	53
Influenza A virus	A/Ross's Goose/Arkansas/AH0085761S.4.A/2016 (H11N9)	N gene	MN253675.1	56	48	40
Influenza B virus	B/Hong Kong/CUHK21967/2000	N gene	MF955545.1	52	40	36
Respiratory syncytial virus	isolate RSVA/USA/ACRI-051/2016	N gene	MN630104.1	47	40	66
Legionella pneumophila	C9_S	N gene	CP015942.1	72	65	60
Haemophilus influenzae	IH197	N gene	MG694561.1	56	47	36
Mycobacterium tuberculosis	FDAARGOS_757	N gene	CP054013.1	84	52	43
Bordetella pertussis	J802	N gene	CP033303.1	80	No Sig.	83
Pseudomonas aeruginosa	PcyII-40	N gene	LR739069.1	60	72	46
Pneumocystis jirovecii	T551_01783	N gene	XM_018374046.1	40	48	56
Staphylococcus epidermidis	SR1	N gene	AF269311.1	52	76	46
Enterovirus	CMC718	N gene	MN629889.1	65	52	50
SARS-	human/EGY/CUNCI-	N	MW547443.1	75	64	76

coronavirus	HGC9I015/2020	gene				
Chlamydia pneumoniae	H12	N gene	LN847142.1	52	48	66
Streptococcus pyogenes	NCTC8231	N gene	LS483345.1	54	68	63
Streptococcus pneumonia	4041STDY6836170	N gene	LS483449.1	43	80	68
Cryptococcus neoformans	B-3501A	N gene	XM_767816.1	60	60	46
Candida albicans	SC5314 Mnn13p	N gene	XM_715485.2	63	44	46
Enterovirus EV68	NIE0611579	N gene	KX162706.1	50	57	56
Rhinovirus	16-J2	N gene	KY629935.1	30	45	48
Mycoplasma pneumonia	NCTC10119	N gene	LR214945.1	54	57	45
Streptococcus salivarius	ICDC3	N gene	CP018189.1	57	65	53
human genome	CHM13	N gene	CP068259.1	53	58	64

- **Cross-reactivity (Clinical Specificity)**

The clinical cross-reactivity of the Stark Sars-CoV-2 Molecular Diagnostic Kit was assessed using a panel comprising various concentrations of negative plasma samples. This evaluation revealed no instances of potential cross-reactivities with pathogens, affirming the high clinical specificity of the assay.

Table 10: Cross-reactivity of the novel Coronavirus (Ncov-2019) resulted from Stark Sars-CoV-2 Molecular Diagnostic Kit setup.

Virus/Bacteria/Parasite	Source/ Sample type	Concentration	Ct Value (ORF1ab gene/N gene)
Adenovirus	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Influenza A	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Influenza B	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Legionella pneumophila	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Cryptococcus neoformans	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Chlamydia pneumonia	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Streptococcus pneumoniae	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Respiratory Syncytial Virus	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Mycoplasma pneumoniae	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Streptococcus pyogenes	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-
Mycobacterium tuberculosis	AmpliRun®DNA/RNA Vircell	104 copies/ml	-/-

10 Pooled human genomes	Clinical sample	10 ng/µl	-/-

- Accuracy

Accuracy assessment encompasses both In Vitro Intra-assay and Inter-assay evaluations.

Intra-assay

Intra-assay refers to the accuracy and precision of the designed method in determining the concentration of similar repeats in one Real-Time PCR cycle. Three repetitions of each concentration of the control sample were examined in one reaction, and coefficient of variation (CV) values were calculated for the threshold cycle (Ct) values. For the N gene, the maximum coefficient of variation is 1.2, and the minimum coefficient of variation is 51%; for the RdRp gene, the maximum coefficient of variation is 1.19, and the minimum coefficient of variation is 29%. All acceptable results must have a CV of less than 5%.

Inter-assay

Inter-assay refers to the reproducibility of results across different runs in Real-Time PCR or results from other laboratories. Five repeats of each concentration of the control sample were tested on three additional days. For the N gene, the maximum coefficient of variation is 2.27, and the minimum coefficient of variation is 0.8; for the RdRp gene, the maximum coefficient of variation is 1.99, and the minimum coefficient of variation is 44%. All acceptable results must have a CV of less than 10%.

- Clinical Evaluation

The clinical performance of the Stark Sars-CoV-2 Molecular Diagnostic Kit was assessed using 185 throat and nasal swab samples collected from patients suspected of COVID-19 in a virus transmission environment. The diagnostic Kit was compared to the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR Fluorescence Probing) from Sansure Biotech Inc., which was granted emergency use authorization by the US Food and Drug Administration. Both methods were performed using Real-Time PCR (Rotor-Gene Q-Qiagen). The results showed a Negative Percent Agreement (NPA) of 100% and a Positive Percent Agreement (PPA) of 97.64%.

Table 11: Clinical evaluation between Stark Sars-CoV-2 Molecular Diagnostic Kit (CARBON Technologies LLC) and Sansure Biotech Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) as Comparator Method

Test type		Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR Fluorescence Probing) (Sansure Biotech Inc)		Total
		Positive	Negative	
Stark Sars-CoV-2 Molecular Diagnostic Kit	Positive	83	0	83
	Negative	2	100	102
Total		85	100	185

- Positive Agreement Rate: 83 ÷ 85 × 100% = 97/64%
- Negative Agreement Rate: 100 ÷ 100 × 100% = 100%
- Overall Rates of Agreement: (98/91% = 100%) × (2 + 100 + 0 + 83) ÷ (100 + 83)

Symbols

	Manufacturer
	Date of manufacture
	Use-by date
	Contains sufficient for <n> tests
	Consult instructions for use
	Biological risks
	Temperature limit
	CE Marking
EC REP	EU Representative
IVD	In Vitro diagnostic medical device
REF	Catalogue Number
LOT	Lot Number

Troubleshooting

For troubleshooting assistance with the Stark Genotyping HPV Molecular Diagnostic Kit, please consult the following guidelines. The CARBON Technologies LLC Technical Support Team is available to address any further questions or concerns you may have.

Problem	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during the preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats and replace test tubes and tips in use.
	Contamination of the extraction/preparation area	Ensure plates are sealed correctly

	PCR tube not properly sealed	
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of RNA samples carrying interferences	Repeat the test with the neat extracted RNA and 1:2, 1:10 dilution of the extracted RNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

Technical assistance

For technical assistance, CARBON Technologies LLC ensures your complete satisfaction. Our technical support team comprises highly trained and experienced scientists adept at troubleshooting most problems you may encounter. They can offer expert advice to help you select the most suitable product for your needs.

You can contact our technical support team anytime through the following methods:

- Phone: +96897058350
- Directly submit your questions to the CARBON Technologies technical support team through our website: www.carbontechnologiesco.com
- Email your questions to: technicalsupport@carbontechnologiesco.com

Rest assured, our team is dedicated to providing prompt and effective assistance to address any inquiries or issues you may have. We are committed to ensuring your satisfaction and success in using our products.

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