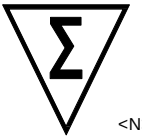
SpectronRx

TAXKO

Hymon™ SARS-CoV-2 Test Kit Instructions for Use (Handbook)



Revision 1

For *in vitro* diagnostic use

Emergency Use Authorization (EUA) Only

Rx Only

IVD In Vitro Diagnostic

REF Catalog # 351251

Distributed by: dba SpectronRx
9550 Zionsville Road, Suite 1
Indianapolis, IN 46268 US

Manufactured by: HymonBio Co. LTD
52 Yingang Rd., Bldg 4, TC-BIOBAY
Suzhou 215434 China

SpectronRx

TAXKO

Content

Intended Use

The Hymon SARS-CoV-2 Test Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable 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 infective status. Positive results do not rule out bacterial co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive 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.

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

Technical Principles

Based on high-efficiency enzyme processing technology and real-time fluorescent quantitative PCR. The lysis process and removal of impurities are achieved in a single tube, and then the nucleic acids were released. The test targets the N and E gene sequences of SARS-COV-2 as amplification target regions. Amplifying with specific primers and fluorescent probes, the test utilizes human genome sequence target as internal quality control.

Warnings and Precautions

For *in vitro* diagnostic use under Emergency Use Authorization only.

1. The Hymon SARS-CoV-2 Test Kit has not been FDA cleared or approved; the test 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, to perform high complexity tests.
2. The Hymon SARS-CoV-2 Test Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
3. The Hymon SARS-CoV-2 Test Kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
4. All samples shall be considered potentially infectious and shall be operated and handled in strict accordance with the laboratory's bio-safety requirements. The experimental personnel should receive professional training (including sample processing, reagent preparation, instrument operation and software setting, etc.). For the laboratory management specifications, please strictly follow the relevant management specifications for gene amplification test laboratories issued by local regulatory agencies.
5. The laboratory should be separated by reagent preparation area, sample preparation area and amplification detection area. All the articles in each area are for special purposes, and they shall not be used for other purposes, as to avoid contamination. The suggested PPE (Personal Protective Equipment) for a laboratory worker are gowns or closed lab coats, hair nets, gloves, eye protection (face shield or goggles) and surgical facemasks or fit-tested N95 masks. Laboratory clothes, hats, shoes, gloves, etc. shall be fully equipped during operation to avoid direct contact of reagents or samples with skin. In case of liquid leakage, wash with plenty of water

immediately. In case of contact with skin wounds, inform local health and epidemic prevention department in time.

6. The real-time fluorescent quantitative PCR analyzer should be calibrated regularly.

7. Please dispose of pipette and kit waste in a designated manner as per company safety policies, and in accordance with local, regional, and federal regulations.

8. Clean the working area immediately after the experiment. The areas and surfaces should be disinfected with 1% sodium hypochlorite, 75% alcohol or UV light regularly.

9. The quality of the test results (for samples and controls) are related to the collection, transportation, treatment and preservation of samples. If any of the sampling, storage, or testing process is improperly executed, this may lead to false negative or false positive results of the test.

Materials Provided

Kit contents

Item	Size	Amount	Contents
Sample reagents			
LY Buffer Solution	500 µL	1 tube	Tris
PN Buffer Solution	50 µL	1 tube	Lysozyme
Nucleic acid detection reagents			
PM Buffer (PCR reaction solution)	1.3 mL	1 tube	Primers & probes, dNTPs, $MgCl_2$
RE Mix (Reverse transcriptase mixture)	100 µL	1 tube	Reverse transcriptase, Taq DNA polymerase
Controls			
PC (Positive control)	25 µL	1 tube	Synthetic RNA
NC (Negative control)	900 µL	1 tube	H_2O

Note:

1. Negative control should participate in nucleic acid extraction as a sample.
2. Positive control does not participate in the extraction of nucleic acid and should be directly added into the PCR reaction solution.

Controls that will be provided with the Hymon SARS-CoV-2 Test Kit include:

Positive Control (PC): Positive Control for the Hymon SARS-CoV-2 Test Kit consists of synthetic RNA of the SARS-CoV-2 N gene and E gene, as well as plasmid of human ACTB gene segment. The concentration of the positive control is 300 copies/mL. 2019-nCOV positive template control is needed to control quality and avoid "false negative" results. The positive control does not participate in the extraction process but should be directly added into the PCR reaction solution for each test.

Negative Control (NC): A "no template" (negative) control, normal molecular grade H_20, is needed to avoid "false positive" results, the negative control is taken through the entire sample processing procedure and should be used for each test.

Internal Control: One heterogeneous internal control is used for process quality and one housekeeping gene (ACTB gene) internal control is used to monitor sampling quality (VIC Channel).

Reagent Storage and Handling

Hymon™ SARS-CoV-2 Test Kit should be stored at -20°C and the reagents are valid for 12 months.

Components Required but Not Supplied in Kit

- ABI 7500 Real-Time PCR System
- Swab specimens with a synthetic tip; such as nylon and an aluminum or plastic shaft
- 1.5 mL DNase-free and RNase-free Eppendorf tube
- 0.2mL PCR tube or strip
- Pipettor and pipette tips (Assorted 10μL, 200μL, 1000μL tips with filters)
- Centrifuge (Can reach up to 12,000 rpm)
- Microcentrifuge
- Vortex mixer
- 0.9% NaCl
- -20°C cold blocks
- DNAZap or equivalent detergent solution
- Bleach
- Disposable gloves and surgical gowns

Specimen Handling, Storage and Preparation

1. The recommended sample type for Hymon SARS-CoV-2 Test Kit is an upper respiratory specimen (such as nasopharyngeal, oropharyngeal, mid-turbinate, or nasal swab) or BAL specimens. Swabs should be a Universal Transport Media (UTM™) or equivalent.

2. Freshly collected samples can be tested immediately or stored at -20±5°C for no more than 3 months. Please avoid freezing and thawing more than three times.

Procedure

Nucleic acid extraction

The below steps should be followed in the order described:

The extractions of nucleic acids in the sample are performed in the sample preparation area.

1. Refer to the table below to prepare master mix according to the actual volume:

One single serving master mix		
Reagent	LY Solution	PN Solution
Volume	4.5 μL	0.5 μL

2. Flush spin after the components are fully mixed so all contents are settled down to the bottom of the tube. Add 45 μL swab eluate/negative control to a 1.5 mL tube, and then add 5 μL master mix in each tube. Vortex the tubes to mix the liquid and then centrifuge.

3. Incubate the tubes containing the samples at 58°C for 10 min, 95°C for 2min.
4. The obtained product can be directly used for PCR reactions.

Note: The negative control in this kit participates in the extraction and is used to monitor the process; the positive control does not participate in the extraction and is used for the quality control of PCR detection reagents.

Nucleic acid amplification and detection

The preparation of PCR reagents is also performed in the reagent preparation area.

1. Take the PCR reaction solution out of the kit, melt at room temperature, vortex and mix well, and flush spin to settle contents to the bottom of tube.
2. Refer to the table below to prepare the PCR mix according to the actual volume:

One single serving PCR mix		
Reagent	PCR reaction solution	Reverse transcriptase mixture
Volume	13 μL	1 μL

3. Flush spin after the components are fully mixed, so that all contents are settled to the bottom of the tube. Add 14 μL prepared PCR mix to each PCR tube.

The sample loading is performed in the sample preparation area.

1. Add 6 μL of the processed negative control, the nucleic acid of the sample to be tested and the positive control into each PCR reaction tube successively. Cover the tubes tight, and mix the tubes thoroughly.
2. Transfer PCR reaction tubes to the amplification detection area after flush spin.

The nucleic acid amplification is performed in the amplification detection area.

1. Put the reaction tubes into the sample tank of the instrument.
2. ABI 7500 settings using software 2.3:

   2.1 Open the "setup" window, set Negative control (NC), Positive control (PC), Un-known samples (Unknown) according to the corresponding order of samples, and set the sample names in the "Sample Name" column.

   2.2 Open the "instrument" window and set the cycle conditions as follows:

   42°C , 5 min;

   94°C , 1 min;

   95°C 15 sec, 60°C 31 sec, 72°C 30 sec;

   The fluorescence signal is collected at 60 °C , and the signal collection channels are FAM and VIC, 40 cycles.

After setting, save the file and run the program.

Interpretation of Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

Acceptable Ct values for Positive and Negative Control

Products of Quality Control	Requirements of Quality Control	
	FAM Channel (Target E, N Gene)	VIC Channel (IC)
Positive Control of 2019-nCoV	Ct ≤ 30	Ct ≤ 30
Negative Control	Undetected	Undetected

Examination and Interpretation of Test Results in each channel

Detection results		Result	Interpretation of Results
FAM Ct value (Target E, N Gene)	VIC Ct value (IC)		
No result or Ct > 38	Ct ⩽ 38	SARS-CoV-2 negative	Indicates that there is no RNA of SARS-CoV-2 in the tested sample or its concentration is lower than the limit of detection with the kit
No result or Ct > 38	Ct > 38	Need reexamination	The result is not valid. The cause shall be researched and eliminated, and the sample shall be rechecked.
Ct ⩽ 38	Ct ⩽ 38 or Ct > 38	SARS-CoV-2 positive	Indicates that the tested sample contains RNA of SARS-CoV-2

Limitations

Assay Limitations

1. The performance of the Hymon™ SARS-CoV-2 Test Kit was established using nasopharyngeal swab samples. Oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs and bronchoalveolar lavage (BAL) specimens are also considered acceptable specimen types for use with the Hymon™ SARS-CoV-2 Test Kit, but performance has not been established.
2. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
3. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction kits have not been evaluated.
4. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.
5. This kit is intended for classification and detection of SARS-CoV-2. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and treatment responses.
6. Although the detected target sequences of this kit are the conservative region of SARS-CoV-2's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.
7. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined.

Conditions of Authorization for the Laboratory

The Hymon™ SARS-CoV-2 Test Kit 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/emergency-situations-medical-devices/emergency-use-authorizations.

However, to assist clinical laboratories using the Hymon™ SARS-CoV-2 Test Kit ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with test result reports, 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 your product will use your product as outlined in the Hymon™ SARS-CoV-2 Test Kit Instructions for Use (Handbook). 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 your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product 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 your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and dba SpectronRx (via email: COVID19info@spectronrx.com or via phone: 1-877-276-1137), any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

G. You, 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] The letter of authorization refers to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

ASSAY PERFORMANCE

Analytical Sensitivity:

To determine the Limit of Detection (LoD) and analytical sensitivity of the Hymon™ SARS-CoV-2 Test Kit, studies were performed using serial dilutions of a previously characterized positive sample for SARS-CoV-2 analyte and the LoD was determined to be the lowest concentration of template that could reliably be detected 95% of all tested positive.

To determine the RNA concentration in these patient samples, plasmids containing SARS-CoV-2 were prepared and quantified with digital PCR.

The LoD was confirmed by testing 20 replicates at the tentative LoD concentration of 5 copies/reaction. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of 20/20.

LoD Study Confirmation Summary

Target	SARS-CoV-2 target in NP
RNA Concentration	1.2 copies/uL
Positives/Total	20/20
Mean Ct	36.42
SD (Ct)	0.68
CV	1.94%

The LoD was confirmed to be 5 copies/reaction based on a positivity rate of ≥ 95% for 20 replicates.

Inclusivity (Analytical sensitivity):

The Hymon™ SARS-CoV-2 Test Kit has been designed to detect all publicly available SARS-CoV-2 viral RNA sequences. As many as possible target sequences were retrieved from GISAID and other publicly available databases and were aligned to identify conserved regions and also specific regions of the SARS-CoV-2 genome where primers and probes were designed for the assay. Alignments were performed with the designed oligonucleotide primer and probe sequences of the Hymon™ SARS-CoV-2 Test Kit with 1659 sequences from the GISAID database.

Cross-reactivity (Analytical Specificity):

The Hymon™ SARS-CoV-2 Test Kit has been designed to detect all publicly available SARS-CoV-2 strains. At the same time, the primers and probes were designed in the SARS-CoV-2 virus specific genome region ensuring the specific detection of the SARS-CoV-2 viral RNA. In silico analysis of the Hymon™ SARS-CoV-2 Test Kit design were performed and compared to common respiratory flora and other viral pathogens from the same genetic family as SARS-CoV-2 according to the Recommended List of Organisms to be analyzed in silico.

For in silico testing, at least 2 strains were tested for each pathogen. The result for in silico cross-reactivity shows that the target genes have low homology with listed organisms, except with some stains of SARS, where the sequences of forward primers and probes showed high homology as presented in the table below. This high homology does not impact actual testing result, because the specificity of the real-time PCR reaction is determined by two primers and a probe and will not affect the overall detection specificity of the Hymon™ SARS-CoV-2 Test Kit.

Cross-Reactivity In-silico

Organism	Homology
SARS-coronavirus **mismatch analysis below*	F1:100% homology with SARS-CoV Consensus R1:65% homology with SARS-CoV Consensus P1:91% homology with SARS-CoV Consensus F2: 94% homology with SARS-CoV Consensus R2: 72% homology with SARS-CoV Consensus P2: 100% homology with SARS-CoV Consensus F3: No alignment found R3: No alignment found P3: No alignment found
MERS- coronavirus	No alignment found
Human coronavirus 229E	No alignment found
Human coronavirus OC43	No alignment found
Human coronavirus HKU1	No alignment found
Human coronavirus NL63	No alignment found
Adenovirus (e.g. C1 Ad. 71)	No alignment found
Human Metapneumovirus (hMPV)	No alignment found
Parainfluenza virus 1-4	No alignment found
Influenza A & B	No alignment found
Enterovirus (e.g. EV68)	No alignment found
Respiratory syncytial virus	No alignment found
Rhinovirus	No alignment found
Chlamydia pneumoniae	No alignment found
Haemophilus influenzae	No alignment found
Legionella pneumophila	No alignment found
Mycobacterium tuberculosis	No alignment found
Streptococcus pneumoniae	No alignment found
Streptococcus pyogenes	No alignment found
Bordetella pertussis	No alignment found
Mycoplasma pneumoniae	No alignment found
Pneumocystis jirovecii (PJP)	No alignment found
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract	No alignment found (for FAM channel) Alignment in human ACTB (for VIC channel)
Candida albicans	No alignment found
Pseudomonas aeruginosa	No alignment found
Staphylococcus epidermis	No alignment found
Staphylococcus salivarius	No alignment found

Microbial Interference Studies:

Microbial interference studies were not performed because *in silico* analysis demonstrated no alignment between the primer/probe design of the Hymon SARS-Cov-2 Test Kit other that SARS-CoV. Free energy analysis indicated that the alignment found in SARS-CoV would not break free energy barrier for PCR primer-pair annealing under the cycling parameters of the test. Therefore, the *in silico* study data predict that no interference would be caused by the microbial pathogens listed above.

Endogenous Interference Substances Studies:

An Interference substance study was performed to evaluate the Hymon™ SARS-CoV-2 Test Kit performance in the presence of interfering substances commonly found in respiratory specimens. Contrived specimens were prepared with the positive control at 3x LoD and spiked with clinically relevant concentrations of interfering substance and ran in triplicate. The results, summarized in the Table below, show no observed interference by the presence of the tested interfering substances.

Substances Tested for Interference

Potential Interferent	Final Concentration	% Agreement with Expected Res	
		Positive Control (3X LoD)	Negative control
Entecavir	0.5 mg/mL	100%（3/3）	100%（3/3）
phenoxymethylpenicillin Potassium Tablets	10 mg/ml	100%（3/3）	100%（3/3）
Azithromycin	1 mg/ml	100%（3/3）	100%（3/3）
Clarithromycin	10 mg/ml	100%（3/3）	100%（3/3）
Mometasone furoate	5 mg/ml	100%（3/3）	100%（3/3）
Fluticasone propionate	5 mg/ml	100%（3/3）	100%（3/3）
Blood (Human)	10% v/v	100%（3/3）	100%（3/3）
Listerine Cool Mint Antiseptic Mouth Wash	10% v/v	100%（3/3）	100%（3/3）
Sputum	n/a	100%（3/3）	100%（3/3）

Clinical Evaluation:

A clinical study was performed to evaluate the performance of the Hymon™ SARS-CoV-2 Test Kit. A total of 76 clinical nasopharyngeal/oropharyngeal swab specimens (31 positive and 45 negative) were compared with a Sansure Biotech Novel Coronavirus (2019-NCOV) Nucleic Acid Diagnostic Kit (EUA200294). The results are summarized in the Tables below. The observed Positive Percent Agreement (PPA) was 100% (97.7% - 100%) detecting 31 out of 31 positive specimens. The Negative Percent Agreement (NPA) was 100% (94.6%-100%) detecting 45 out of 45 negative specimens.

Hymon™ SARS-CoV-2 Test Kit	Sansure Biotech Novel Coronavirus (2019-NCOV) Nucleic Acid Diagnostic Kit Comparator		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	31		31	PPA 100%	97.7-100%
Not Detected		45	45	NPA 100%	94.6-100%
Total	31/31	45/45	76		

Symbols

The following table describes the symbols that may appear on the labeling or in this document.

Σ <N>	Contains reagents sufficient for <N> reactions
	Use by
IVD	*In vitro* diagnostic medical device
REF	Catalog number
	Manufacturer
Rx Only	For Prescription Use Only

Ordering Information

Product	Contents	Cat. no.
Hymon ™ SARS-CoV-2 Test Kit	For 96 tests	351251

Distributed by:
dba SpectronRx
9550 Zionsville Road, Suite 1
Indianapolis, IN 46268

Contact Number and/or Email:
1-877-276-1137 and/or
COVID19info@spectronrx.com
www.spectronrx.com

Manufactured by:
HymonBio Co. LTD
52 Yingang Rd., Bldg 4, TC-BIOBAY
Suzhou 215434 China

For up-to-date licensing information and product-specific disclaimers, see the respective dba SpectronRx kit handbook or user manual. dba SpectronRx kit handbooks and user manuals are available at www.spectronrx.com or can be requested from dba SpectronRx Technical Services or your local distributor.

Revision History	Date	Change Control
Revision 1	05/2020	Original release.