ADALTIS

MOLgen

SARS-CoV-2 Real Time RT-PCR Kit

REF MESARS-CoV-2

Σ 100 rxns

-15°C -25°C

IVD

CE

This package insert must be read carefully before product use.
Package insert instructions must be carefully followed.
Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Manufacturer:
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en

SYMBOLS USED ON LABELS							
English EN	IVD	CE	REF	LOT			
	In Vitro Diagnostic Medical Device	CE Mark	Catalogue Number	Lot Number	Attention, See Instructions For Use	Temperature Limitation	Use By
	Σ				MASTER MIX	PROBE MIX	POS CTRL
	Number of Reactions	Manufacturer	Date of Manufacture	Keep away from Sunlight	Master Mix	Probe Mix	Positive Control
	NEG CTRL						
	Negative Control						

Legals:

Limited Product Warranty:
This warranty limits our liability for the replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Adaltis shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

EN *ENGLISH*

A. INTENDED USE

MOLgen SARS-CoV-2 Real Time RT-PCR Kit is used for the qualitative detection of Novel Coronavirus (SARS-CoV-2), by Reverse Transcription (RT) and Real Time Polymerase Chain Reaction (PCR) from RNA extracted from human respiratory specimens such as nasopharyngeal swabs, oropharyngeal swabs, sputum and bronchoalveolar lavage fluid (BALF).

B. INTRODUCTION

Coronaviruses are a large family of respiratory viruses, some causing illness in human and others circulating among animals such as camels, cats and bats from common cold to severe diseases such as Middle Est Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) is a new strain of coronavirus emerged from Wuhan, China in December 2019.

The infection with SARS CoV2 virus causes Coronavirus disease (COVID-19) where "CO" stands for crown, "VI" for viruses, "D" for disease and “19"' indicates the year in which it occurred.

COVID-19 symptoms are fever, cough , pneumonia and Severe Acute Respiratory Syndrome (SARS).

C. PRINCIPLE OF THE TEST

MOLgen SARS-CoV-2 Real Time RT-PCR Kit is intended for the detection of the New Coronavirus SARS-CoV-2 RNA using reverse transcription of viral RNA and real-time polymerase chain reaction (RT-PCR) method with fluorescence detection of amplified product.

Real-time PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is a dual-labeled DNA-probe that specifically binds to the target region of the pathogen’s cDNA. Fluorescence signal increases due to the separation of fluorescence dye and quencher owing to Taq DNA-polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of cDNA, primer annealing and complementary chain synthesis.

Threshold cycle value (**Ct**) is a cycle number, at which the fluorescence generated within a reaction crosses the threshold and the fluorescence signal rises significantly above the background. The increased signal is due to the use of a DNA hybridization probe that is specific for the given cDNA sequence: it binds to the cDNA in the course of reaction and provides additional specificity of the method. The DNA probe consists of a fluorescence dye at the 5’-end and a fluorescence quencher at the 3’-end that significantly reduces the fluorescence intensity. During the polymerase synthesis of the complementary strand, the probe is cleaved from the 5’-end due to the 5’-3’ nuclease activity of Taq DNA polymerase, the quencher and the dye become separated thus increasing the fluorescence signal due to accumulation of the reaction product. The detected fluorescence intensity depends on the initial quantity of pathogen’s cDNA template in the sample.

The primer and probe set is designed to detect SARSCoV2 N gene, E gene and RdRP gene.

The detection of amplified virus RNA fragment is performed in fluorimeter **channel FAM**, **ROX and CY5**. The use of Internal Control (IC) on **HEX** channel ensure the monitoring correct amplification of the samples.

The results of PCR analysis are taken into account in complex diagnostics of disease. The kit is validated for use with: AMPLIlab (Adaltis), CFX96 (Bio-Rad), RotorGene Q (Qiagen).

Applications

Kit for Novel Coronavirus (SARS-CoV-2) detection using real-time PCR based on the use of Dual Probes.

D. COMPONENTS

The kit contains reagents required for 100 reactions.

Component Code	Component Description	Nr Vial	Volume (µL/vial)	Lip Color
MESARSCoV2/MM	MOLgen SARS-CoV-2 Master Mix	1	1000	Green
MESARSCoV2/PM	MOLgen SARS-CoV-2 Probe Mix	1	500	Yellow
MESARSCoV2/PC	MOLgen SARS-CoV-2 Positive Control	1	50	Red
MESARSCoV2/NC	MOLgen SARS-CoV-2 Negative Control	1	50	Clear
Number of reactions (rxns)			100	
Reference Kit Code			MESARS-CoV-2	

1. MOLgen SARS-CoV-2 Master Mix

Ready to use reagent.

It contains reagents for reverse transcription and amplification.

2. MOLgen SARS-CoV-2 Probe Mix

Ready to use reagent.

It contains primers and probes which are premixed to the working concentrations.

3. MOLgen SARS-CoV-2 Positive Control

Ready to use control.

It contains plasmids with SARS-CoV2 sequences (RdRP, E and N genes) plus an Internal Control (IC).

4. MOLgen SARS-CoV-2 Negative Control

Ready to use control.

Negative Control should be included at every run as it indicates that reagents have not been contaminated.

E. MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Nucleic Acid extraction kit intended for isolation of RNA.
- Real-time PCR cycler
- Biosafety Cabinet
- Refrigerator
- Microcentrifuge with rotor for 1,5 mL reaction tubes
- 1.5 mL microcentrifuge tube for extraction
- Plate shaker
- Calibrated adjustable pipettes
- Disposable pipette tips with aerosol filter
- Strip Tubes and Caps (0.2 mL) or PCR Tubes (0.2 mL) or 96 well plate with optical caps or films.
- Disposable medical non-sterile powder-free gloves
- Magnetic rack for tubes
- Biohazard waste container

NOTE: The extraction protocol with MOLgen Universal Extraction Kit (Adaltis) Ref. ME188830 and the PCR set-up procedure can be done in automated mode using the Adaltis EXTRAlab Instrument by executing the extraction and PCR set-up protocol preloaded in the instrument user interface

F. WARNING AND PRECAUTIONS

- This kit has to be used by skilled and properly trained technical personnel only, under the supervision of a medical doctor responsible of the laboratory.
- To obtain reliable results, strictly follow this Instruction for use provided with the kit.
- Read carefully the Safety Data Sheet (SDS) before product use.
- Do not use the components from the Kits of different lots.
- For components avoid more than 3 thawing- freezing cycles.
- All the personnel involved in performing the assay have to wear protective laboratory clothes, talc-free gloves and glasses.All personnel involved should be trained in biosafety procedures, as recommended by the Center for Disease Control, Atlanta, U.S. and reported in the National Institute of Health's publication: "Biosafety in Microbiological and Biomedical Laboratories".
- When handling the kit, follow the national safety requirements for working with pathogens.
- Treat all specimens as potentially infective using safe laboratory procedure. Refer to Interim Laboratory Biosafety Guidance for Handling and Processing Specimens Associated with 2019-nCoV.
- To prevent cross-contamination it is recommended to have dedicated areas for sample preparation (RNA extraction) and PCR test (setting up PCR reaction and thermocyclers).
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- To conduct real-time amplification reaction with PCR products detection, use only disposable tips with aerosol filters.
- Do not pool reagents from different lots or from different vials of the same lot.
- Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- Do not use the kit after the expiration date.
- Do not smoke,drink eat or apply cosmetics in areas in which specimens or Kit components are handled.

G. SPECIMENS COLLECTION AND STORAGE

- Collect fresh specimen of Nasopharyngeal swabs, Oropharyngeal swab, Sputum and BALF from suspects. Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coranovirus (2019 nCoV).
- For collection methods refer to specimen collection devices manufacturer instructions.
- For the collection of the sample, the manufacturer of the kit MOLgen SARS-CoV-2 Real Time RT-PCR, recommends the use of the collection system in liquid transport medium.
- **Nasopharyngeal-Oropharyngeal swabs**: Carefully take out the swab from package and quickly rotate it around two sides of fauces, throat and tonsila few times applying pressure to collect as much secretions as possible. Avoid touching tongue. Break the swab stick and put the head into sampling solution in specimen tubes. Screw the tube cap tightly to ensure no leakage.
- **Sputum**: Have the patient rinse their mouth with plain water or normal saline. Deep breath and bring the thick secretions from the lungs rather than expectorating saliva or the thin secretions from the mouth and Nasopharynx. Collect the sputum into sterilized container and seal it to deliver for test.
- **BALF:** Collect 3 ml of unprocessed BALF in sterile, dry and clean DNase/RNase free Cryotubes. Screw the tube cap tightly to ensure no leakage and seal the tube with film.

Specimens can be stored at 2…8℃ for up to 72 hours after collection or at -70℃ for longer periods.

H. SAMPLE EXTRACTION

The assay is performed on extracted RNA specimens obtained from the clinical material using commercial Nucleic Acid Extraction Kit intended for isolation of RNA. Using the "Molgen Universal Extraction Kit" Ref. ME188830 the recommended Final Elution Volume is 100 µL.

Specimen can be used for analysis:
Nasopharyngeal extracts, Oropharyngeal extracts, Deep cough sputum, BALF.

Extracted RNA cannot be stored! Perform RNA extraction from clinical material immediately before running the RT-PCR

I. PROCEDURE

- Prepare the Reaction Mix of the following reagents taking into consideration the number of samples, 1 Positive Control and 1 Negative Control

Component	1 rxn Volume required	N rxns * Volume required
MOLgen SARS-CoV-2 Master Mix	10 µL	N rxns x 10 µL
MOLgen SARS-CoV-2 Probe Mix	5 µL	N rxns x 5 µL

*N rxn = total samples + 1 PC + 1 NC + 1 extra

- Pipette 15 µl of Reaction Mix on each tube of sample, Positive Control and Negative Control.
- Add 5 µl of extracted RNA for Negative Control.
- Add 5 µl of extracted RNA for each samples and Positive Control in dedicated area.
- Seal the reaction tubes with appropriate lids.
- Label the reaction tubes with Reaction MIX for each specimen and control sample.

An example of dispensation scheme is reported in the table below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S2	S5									
B	NC	S3	S5									
C	NC	S3	S6									
D	S1	S3	S6									
E	S1	S4	S6									
F	S1	S4	PC									
G	S2	S4	PC									
H	S2	S5	PC									

Legenda:
NC = Negative Control
S = Extracted Sample
PC = Positive Control

- Place the reaction tubes into the Real-Time PCR cycler.
- Program the Real-Time PCR cycler as follows

Steps	Cycles	Temperature	Time
Reverse Transcription	1	45°C	10 minutes
Enzyme Activation	1	95°C	2 minutes
Amplification	40	95°C	5 seconds
		60°C (*)	25 seconds

(*) Measure the fluorescence at 60°C on FAM, ROX, CY5, HEX channels.

- Select the amplification detection channels:

  Collect data through the "FAM" channel for the detection of amplification signal of **RdRP gene**.

  Collect data through the "ROX" channel for the detection of amplification signal of **E gen**e.

  Collect data through the "CY5" channel for the detection of amplification signal of **N gene.**

  Collect data through the "HEX" channel for the detection of amplification signal of **IC.**
- Program the positions of the tubes with the specimens, PC and NC according to the Instruction Manual for the cycler in use.
- Run the program.

L. DATA ANALYSIS AND INTERPRETATION

For basic inform ation regarding data analysis on specific real-time PCR instruments please refer to the user manual of the respective instrument.

Thresholds setting:

Thresholds line must be setted above any background signal.

Validity of Run:

Before interpreting sample results check that both Positive and Negative Controls passed.

For Positive Control the program should:

- detect the increase in RdRP gene cDNA amplification signal on **"**FAM**"** channel at ≤35 Ct.
- detect the increase in E gene cDNA amplification signal on **"**ROX**"** channel at ≤35 Ct.
- detect the increase in N gene cDNA amplification signal on **"**CY5**"** channel at ≤35 Ct.
- detect the increase in IC amplification signal on "HEX**"** channel at ≤40 Ct.

For Negative Control the program should:

- No fluorescence increase should appear on "HEX" channel for IC amplification.
- No fluorescence increase should appear on "FAM" channel for RdRp gene cDNA amplification.
- No fluorescence increase should appear on" ROX" channel for E gene cDNA amplification.
- No fluorescence increase should appear on "CY5" channel for N gene cDNA amplification.

Control Type	FAM	ROX	CY5	HEX
Positive Control Ct value	Ct ≤ 35	Ct ≤ 35	Ct ≤ 35	Ct ≤ 40
Negative Control Ct value	Undetected	Undetected	Undetected	Undetected

Samples analysis and Interpretation of results:

Once the Controls have passed, the unknown samples can be interpreted based on the following table:

FAM	ROX	CY5	HEX	RESULTS
RdRP gene	E gene	N gene	IC	
Undetected	Undetected	Undetected	**Ct 25-40** or Undetected	Negative or below detection limit
CT≤ 40	**CT≤ 40**	**CT≤ 40**	**Ct 25-40** or Undetected	Positive to SARS-CoV-2
Ct ≤ 40	Undetected	**Ct ≤ 40**	**Ct 25-40** or Undetected	Positive to SARS-CoV-2
CT≤ 40	**CT≤ 40**	Undetected	**Ct 25-40** or Undetected	Positive to SARS-CoV-2
RESULT NOTE WHO 19-03-2020* (*) WHO - Laboratory testing for coronavirus disease (COVID-19) in suspected human cases – Interim Guidance 19 march 2020				
Laboratory confirmation of cases by NAAT in areas with no known COVID-19 virus circulation				
A positive NAAT result for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (RdpR and ***E gene or N gene***)				
Laboratory-confirmed case by NAAT in areas with established COVID-19 virus circulation				
In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which, for example, screening by rRT-PCR of a single discriminatory target is considered sufficient. (*E **gene; N gene***)				
CT≤ 40	Undetected	Undetected	**Ct 25-40** or Undetected	Inconclusive
Undetected	Undetected	Undetected	Undetected	PCR inhibition or unsuitable sample. Analyze another sample

Assay Limitations

- Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.
- A false negative result may occur in the sample due to low load (below the product detection limit), improper collection transport or handling of the sample, presence of RT-PCR inhibitors.
- Although efforts have been made to design the RT-PCR assay in conserved regions of the viral genomes. However mutations leading to reduced assay performance and possible false negative results could occur due to the considerable variability of viral genomes.
- Do not use any reagent past the expiration date
- False Positive result may occur due to the several reasons, most of which are connected with RNA contamination during specimen handling and preparation.
- Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system is required.
- All positive results have to be reported to the appropriate public health authorities.
- *Diagnosis of COVID-19 infection has to be taken and released to the patient by a suitably qualified medical doctor.*

M. PERFORMANCE CHARACTERISTICS

ANALYTICAL SENSITIVITY
(Limit of Detection LoD)

The Analytical Sensitivity, defined as the lowest concentration of analyte that could be reliably detected with 95% confidence, of MOLgen SARS-CoV-2 RNA RT-PCR Kit was assessed testing 3-fold serial dilution of RNA samples with 20 extracted replicates.
Analytical Sensitivity was found ≤10 copies/rxn.

ANALYTICAL SPECIFICITY (Cross-Reactivity)

Analytical Specificity was in vitro assessed testing related pathogens from the same genetic family and high priority organism likely in the circulating area.
Materials used:
NATtrol™ Respiratory Verification Panel Catalog Number NATRV2-BIO contains 22 viral and bacterial targets plus 1 Negative Control.

NATtrol ™ Respiratory Verification Panel		
Organism	**Strain**	**Interpreted Results**
Influenza A H1N1	A/New Caledonia/20/99	Undetected
Influenza A H3	A/Brisbane/10/07	Undetected
Influenza A 2009 H1N1	A/NY/02/09	Undetected
Influenza B	B/Florida/02/06	Undetected
Metapneumovirus 8**	Peru6-2003	Undetected
Respiratory Syncytial Virus A	N/A	Undetected
Rhinovirus 1A	N/A	Undetected
Parainfluenza virus Type 1	N/A	Undetected
Parainfluenza virus Type 2	N/A	Undetected
Parainfluenza virus Type 3	N/A	Undetected
Parainfluenza virus Type 4	N/A	Undetected
Adenovirus Type 3	N/A	Undetected
M.pneumoniae	M129	Undetected
C. pneumonia	CWL-029	Undetected
C. pertussis	A639	Undetected
Adenovirus Type 31	N/A	Undetected
Adenovirus Type 1	N/A	Undetected
B. parapertussis	A747	Undetected
Coranovirus NL63	N/A	Undetected
Coronavirus 229E	N/A	Undetected
Coranovirus OC43	N/A	Undetected
Coronavirus HKU-1	N/A	Undetected
Negative	N/A	Undetected

NATtrol™ MERS-CoV Stock Catalog Number NATMERS-ST

NATtrol ™ MERS CoV Stock		
Organism	**Strain**	**Interpreted Results**
MERS-CoV	Florida/USA-2 Saudi Arabia_2014	Undetected

DIAGNOSTIC SENSITIVITY

Diagnostic Sensitivity of MOLgen SARS-CoV-2 Real Time RT-PCR kit have been confirmed in a study conducted on a total number of 16 Confirmed Positive Specimens.

	Positive
MOLgen SARS-CoV-2 Real Time RT-PCR Kit	16
Reference PCR Method	16
Sensitivity	100%

ACCURACY

Accuracy of MOLgen SARS-CoV-2 Real Time RT-PCR kit have been confirmed in a screening study conducted on a total number of 133 Italian specimens including 9 Positive and 124 Negative Confirmed.

	Specimens Status	
	Positive	**Negative**
MOLgen SARS-CoV-2 Real Time RT-PCR Kit	9	124

N. STORAGE AND TRANSPORTATION

- Transport the kit at -15℃ …-25°C.
- Store the kit in the manufacturer's packaging at -15℃ …-25°C for the entire shelf life.
- Do not freeze the Kit more tha 3 times.

O. WARRANTY

The manufacturer hereby guarantees the conformity of manufactured products to the requirements of normative and technical documentation.

Safety and quality of products is guaranteed throughout the entire shelf life.

The manufacturer is responsible for product's unsatisfactory features, except for the defects that have arisen as a result of violation of the instructions of use, transportation and storage conditions, actions of third parties or force majeure.

The manufacturer shall at its own expense replace the product, technical and functional characteristics (consumer properties) of which do not comply with the normative and technical documentation in case these disadvantages were caused by latent defect in material or defective manufacturing.

P. REFERENCES

1. LU Rou-jian, ZHANG Ling-lin, TAN Wen-jie, ZHOU Wei-min, WANG Zhong, PENG Kun, RUAN Li. Development and Comparison of Real- Time and Conventional RT-PCR Assay for Detection of Human Coronavirus NL63 and HKU1[J]. CHINESE JOURNAL OF VIROLOGY,2008(4).
2. NIU P, LU R, LAN J, LIU G, WANG W, TAN W. Development of Novel Multiplex Real-time RT-PCR Assays for Detection of MERS-CoV Infection[J]. CHINESE JOURNAL OF VIROLOGY, 2016(3).
3. CHEN Yu-jing. Development of two-panel reactions of real-time PCR for detection of 18 types/subtypes of respiratory viruses[D]. 2015.
4. CDC. (2020, Feb 6). 2019 Novel Coronavirus > About 2019 Novel Coronavirus (2019-nCoV). Retrieved Feb 7, 2020, from Centers for Disease Control and Prevention: https://www.cdc.gov/coronavirus/2019-ncov/about/index.htlm

Claims regarding the quality of the kit should be addressed to:

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