SARS-CoV2

Qualitative detection of SARS-CoV2 genome

REAL-TIME PCR

100 tests

Instructions for users

REF. **HV034**

VER. **1.0**

CE IVD

INDEX

1. GENERAL INFORMATION

1.1 Intended use

*The **GENESPARK SARS-CoV2*** kit is a rapid, sensitive, specific and reproducible In Vitro Diagnostic Assay for detecting 2019-Novel Coronavirus (SARS-CoV2) virus genome. The kit is intended to be used on viral RNA extracted from whole blood, plasma, serum, nasal and orofaringeal swabs, sputum, broncho-alveolar-lavage (BAL), and other biological fluids. Thanks to its sensitivity and reproducibility, the kit can be employed for general identification purpose of SARS-CoV2 genome.

1.2 Pathology

Novel Coronavirus 2019, abbreviated as SARS-CoV2, is a new strain of coronavirus found in humans in 2019. The general symptoms caused by the virus are fever, fatigue, dry cough, gradually emergence of breathing difficulties, severe symptoms of acute respiratory distress syndrome, sepsis shock, difficult to correct metabolic acidosis and coagulopathy. The virus is able to spread from person to person, the incubation period of the virus is generally 1 to 14 days, and the incubation period is contagious.

SARS-CoV2 is closely related to the original SARS-CoV. It is thought to have a zoonotic origin. Genetic analysis has revealed that the coronavirus genetically clusters with the genus *Betacoronavirus*, in subgenus *Sarbecovirus* (lineage B) together with two bat-derived strains. It is 96% identical at the whole genome level to other bat coronavirus samples (BatCov RaTG13). In February 2020, Chinese researchers found that there is only one amino acid difference in certain parts of the genome sequences between the viruses from pangolins and those from humans, however, whole-genome comparison to date found at most 92% of genetic material shared between pangolin coronavirus and SARS-CoV2, which is insufficient to prove pangolins to be the intermediate host.

The standard method of testing is real-time reverse transcription polymerase chain reaction (rRT-PCR). The test is typically done on respiratory samples obtained by a nasopharyngeal swab, however a nasal swab or sputum sample may also be used. Blood tests can be used, but these require two blood samples taken two weeks apart and the results have little immediate value. Chinese scientists were able to isolate a strain of the coronavirus and publish the genetic sequence, so laboratories across the world could independently develop polymerase chain reaction (PCR) tests to detect infection by the virus.

1.3. Principle of the assay

The ***GENESPARK SARS-CoV2*** kit is based on a Real-Time PCR chemistry including the use of primers and fluorescent multiple probes based on a reporter-quencher system for the identification of a specific sequence of viral RNA. The use of dual - label probes, together with the oligonucleotide pairs functioning as primers for the PCR, allows to monitor the reaction in progress as well as the increase in fluorescence for each sample at the end of the extension phase of each PCR cycle.

For each probe, the 5'-end is labeled with a fluorophore, while the 3'-end is labeled with a quencher. When the probe is intact, the fluorescence emitted by the fluorophore is absorbed by the quencher, and no fluorescent signal is detected.
However during the amplification of the template, the probe will be degraded due to the 5'-3' exonuclease activity of Taq DNA polymerase, and the fluorescent reporter and the quencher are cleaved and separated, then a fluorescent signal can be detected. The generation of each molecular amplicon is accompanied by the generation of a fluorescent signal. Realtime monitoring of the entire PCR process can be assessed by monitoring the accumulation of fluorescent signals.
This product provides triplex-detections in a single tube, including two independent genes of SARS-CoV2 and an internal control which targets the human RNAse P (RNP) gene to assess specimen quality. Specific primers and probes were designed for the detection of conserved region of SARS-CoV2 ORF1ab gene and N gene, respectively, avoiding non-specific interference of SARS2003 and BatSARS-like virus strains.
Internal control (RNSase P gene) provides a nucleic acid extraction procedural control and a secondary negative control. Positive control (SARS-CoV2-RNA target) provides a nucleic acid extraction and a reverse transcription control to validate the entire procedure and reagent integrity.

2. KIT CONTENT

The kit contains reagents and controls for analyzing up to 100 biological samples, (Table 1). The **Reaction Mix** contains the components (buffer, salt, nucleotides, primers and probes) for the amplification and detection of the SARS-CoV2 fragment and the internal control in a single reaction. The **Enzyme Mix** is a stabilized preparation of a Retro-Trancriptase/Hot-Start DNA Taq Polymerase enzyme blend. The **No Template Control (NTC)** serves as negative control for excluding contamination of the reaction leading to false positive results. The **Positive Control (PC)** contains the target RNA of the selected genes of SARS-CoV2. The **Internal Control (IC)** contains artificial RPN gene - RNA target, for use in particular settings (e.g., in samples not containing human nucleated cells)

Table 1 – Kit Content

COLOR CODE	DESCRIPTION	AMOUNT
Green	**Reaction Mix**	5 x 500 µL
Red	**Enzyme Solution**	1 x 150 µL
Blue	**Positive Control (PC)**	1 x 400 µL
Yellow	**Internal Control (IC)**	5 x 250 µL
White	**No Template Control (NTC)**	1x 500 µL

Note: nucleic acid extraction from samples must be performed simultaneously with the extraction of the Positive control (SARS-CoV2 RNA target) and No Template Control (DEPC-Treated Water) for monitoring the entire procedure to reduce false negative or false positive rates.

3. STORAGE INSTRUCTIONS

The *GENESPARK SARS-CoV2* kit is shipped at 4°C (ice packs for 4 days) and must be stored at -20°C. The kit is stable up to the expiration date indicated on the kit label. For a correct use, please follow the following instructions strictly:

- Store the kit at -20°C since the arrival until used, avoiding repeated thawing of reagents during use
- Thaw the amount of reagents not exceeding the assay need
- After the first thawing, Calibrators and Reaction Mix not used in the assay must be stored at 4°C and can be reused **within three months** if correctly stored at 4°C. For prolonged storage exceeding three months, maintain the Calibrators and the Reaction Mix at – 20°C. The Total Mix (Reaction Mix + Enzyme Solution) not completely used in a run can be stored at 4°C and re-utilized **within one month** if correctly stored at 4°C
- The Enzyme Solution must be stored at - 20°C

4. MATERIALS REQUIRED BUT NOT PROVIDED

- DNA Extraction Kit
- Desktop minicentrifuge
- Biological cabinet BLS2
- Filtered tips (aerosol barrier)
- Vortex mixer
- Pipettors (capacity 1-20 µL, 50-200 µL, 200-1,000 µL)
- Tube racks
- Polypropylene sterile tubes
- Disposable gloves, powderless
- Real Time PCR thermalcycler

5. WARNINGS AND PRECAUTIONS

1. This product is for In Vitro Diagnostic Use only.
2. Carefully read all instructions for use and the material safety datasheet in attachment before starting to use the kit.
3. The product is suited to be used with the listed instruments only (see chapter four).
4. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterwards.
5. Do not pipette by mouth.

6. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
7. Do not use the kits after their expiry date. Store the reagents as reported in the instructions for use.
8. The kit can be used by qualified personnel only.
9. Dispose all specimens and unused reagents as potentially infectious, according with local regulation.
10. Samples, materials, reagents and effluents used or come into contact with biological material must be handled as potentially infectious. Make reference to the material safety datasheet for their disposal.
11. Materials containing or suspected of containing infectious agents must be manipulated within microbiological safety rooms and under a Biohazard biological cabinet. The text of "Biosafety Level 2" and of analogous microbiology safety rules are provided upon request.
12. Clean and disinfect all spills of specimens or reagents using a disinfectant, such as 0,5% sodium hypochlorite, or other suitable disinfectants.
13. Avoid contact of specimens and reagents with skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice.
14. Samples from patients under treatment may interfere with the diagnosis.
15. Warnings and precautions for molecular biology:
    - molecular diagnostics (MDx) assays require skilled personnel, for avoiding common errors and manipulations possibly leading to nucleic acid degradation, or contamination of samples and reagents with previously amplified products
    - it is recommended to divide the working area into separate areas for executing the distinct procedure steps: DNA extraction, reagent and mixes setup, dispensing of samples, and amplification
    - manage the workflow in order to avoid contact and transfer of materials, tools, reagents, devices between these separate areas. Each area should be equipped with its own tools and materials tube used only inside that area.
    - dedicated labcoats, gloves, pipettors, tips and other disposables must be provided and mantained in each separated area, avoiding transferring them into other areas
    - organize the work flow in an one-way direction, so that personnel run the assay starting from the extraction area toward the amplification area in successive steps
    - manipulate samples, reagents and mixes under laminar flood cabinets equipped with UV lamps
    - possibly avoid to open different samples tubes at the same time
    - use only positive-displacement or filtered tips
    - after the amplification step, avoid to open tubes containing amplified products. This procedure is unnecessary for real time -based assays and can only lead to contamination with amplicons. If necessary for other purposes,

open the tubes in areas where the risk of contamination by carry over is minimal or absent
- avoid using samples intended for molecular diagnostics for other purposes or other assays.
- avoid to use specimens already processed for other non-MDx tests
- conserve extracted DNA exceeding the use for the test at - 20°C until possible further use or for retesting

6. PROCEDURE OF THE ASSAY

Sampling and handling

1. Suitable specimen type: upper respiratory specimen (including nasal swabs, nasopharyngeal swabs/aspirates/washes and sputum) and lower respiratory specimen (including respiratory aspirates, bronchial washes, broncho-alveolar lavage fluids and lung biopsy specimen).
2. For detailed methods of specimen collection, please refer to the protocol in the "Microbiology Specimen Collection Manual".
3. The collected specimen should be used for detection within the same day. Otherwise, please store the specimen as follows:
Store at 2°C-8°C for no more than 24 hours;
Store at <-20°C for no more than 10 days;
Store at <-70°C for long term, avoinding repeated freeze-thaw cycles.
4. The specimen should be transported using sealed foam box with dry ice.

6.1. RNA Extraction

Samples preparation (Specimen Preparation Area)

The extraction has to be realized accordingly to the corresponding requirements and procedures of viral RNA extraction kits *(Ref. Immunospark extraction kit EXMB003)* . Each nucleic acid extraction procedure must be performed simultaneously with one Positive Control and one Negative Control (adding 5 ul, diluting with sterile saline solutions to desired volume).
The extracted RNA can be directly used for detection. If the extracted RNA is not used immediately for detection, please store it at -70°C, avoiding repeated freeze-thaw cycles. Qiagen, Macherey-Nagel and Promega kit suited for the samples indicated have been successfully used with this assay as well as Immunospark extraction kit *EXMB003*. Contaminants or RNA degradation may significantly affect the assay performances. Avoid to use heparinized blood, as heparin interferes with RNA polymerase activity.

6.2. Amplification reaction set up (Reagents preparation)

- Start the thermalcycler and the software available on the computer dedicated to the thermalcycler.
- Thaw only the aliquots which are necessary for the run (please consider also the volume of reagents for the amplification of the Controls)
- Centrifuge **Reaction Mix** and **Enzyme Mix** at 1,000-3,000 rpm for 5 sec.

- Always use **Positive Control (PC)** and the **Negative Control (No Template Control, NTC)** in parallel to the samples to be tested.
- Prepare a number of 0.2 mL reaction tubes (use the reaction tubes for the optical analysis recommended by the manufacturer of the thermalcycler) corresponding to the number of samples to be tested and add, in each run, 2 tubes (one for the **Positive Control** and one for the **Negative Control - NTC**).
- Prepare a 1.5 mL or 2 mL tube for the Total Mix

Users of Applied Biosystems thermalcyclers (ABI) can add the ROX Reference Dye (0.5 µl/tube in the analytical session) to the Total mix. Add 17 µL Total Mix

NOTE: The ready-to-use Rox Reference Dye can be provided by IMMUNOSPARK separately from the kit.

Follow the scheme below for the preparation of the Total Mix (Table 2) in RNase free environment:

Table 2 – Preparation of the Total Mix

Reaction Mix	**250 µL**
Enzyme Solution	**10 µL**
Total Mix	**260 µL**

Calculate the number of reaction tubes (Sample number + positive control + NTC). It is recommended to set both negative and positive controls for each test.
Mix the above mixture throughly, and make aliquots of 15 µL into different PCR reaction tubes. Then, move to the specimen Preparation area.

Template addition (Specimen Preparation Area)
Add 10 µL of extracted Negative Control products, 10 µL of extracted Positive Control products and 10 µL of extracted RNA from specimen to different PCR reaction tubes which contained 15 µL of PCR mix. The total volume is 25 µL. Cap the reaction tubes tightly, centrifuge them at low speed. Then, move to the detection Area.
Operate according to following scheme (Table 3):

Table 3 – Reaction set up

	Sample	PC	NTC
TOTAL MIX	**15 µL**	**15 µL**	**15 µL**
Sample DNA	**10 µL**		
PC		**10 µL**	
NTC			**10 µL**
TOTAL	**25 µL**	**25 µL**	**25 µL**

6.3 Amplification program and thermal profile set up*

*** these instructions refer to Bio-Rad instruments. For other instruments, please see the producers instruction for use**

Plate set up

- set the command on **Plate** window to modify the plate
- program the plate as follows:
  - *indicate the samples as **Unknown**
  - *indicate the Positive Control as **PC**
  - *indicate the Negative Control (NTC) as **NTC**
  - *select for each sample and control the 2 fluorophore channels as:
    - FAM (*ORF1ab gene* probe)
    - Cy5 (N gene probe)
    - HEX/VIC (IC probe)
  - * indicate the reaction volume as 25 µL
- start the reaction by selecting the command **RUN**. The program automatically.

Set the internal reference parameter to fluorescence of the instrument to "none".

Thermal profile set up (RT-PCR Amplification)

By selecting the command protocol it is possible to recall memorized protocols and to use them for the run to be started, or alternatively to create a new protocol. For using an archivial program dedicated to a kit profile you need simply to select it. The profile recalled will be highlighted as **Selected Protocol**.

Put the reaction tubes in the PCR instrument, setup and run the following thermal profile for the ***GENESPARK SARS-CoV2*** kit is reported in Table 4.

Table 4 - Amplification protocol

STEP	PHASE	CYCLE	TIME	TEMPERATURE (°C)
1	Reverse Transcription	1	15 min	50.0°C
1	Pre-denaturation	1	30 sec	95.0°C
2	PCR cycles	45	10 sec	95.0°C
			30 sec	58.0°C (plate read)

The reaction volume is **25 µL.**

6.4 Results analysis (refer to the instrument user manual)

The result is valid if all the following criteria are met. Otherwise, the result is INVALID:

Table 5 – Interpretation of fluorescence patterns

CTRL	CHANNEL	NORMAL Ct
Negative IC	FAM	No Ct or Ct>33
	Cy5	No Ct or Ct>33
	HEX/VIC	No Ct or Ct>33
Positive IC	FAM	Ct≤33
	Cy5	Ct≤33
	HEX/VIC	Ct≤33

6.5 Interpretation of the results

If the criteria of QUALITY CONTROL is met, analyse the data of samples as follows:

1. If the Ct value of HEX/VIC (IC) channel is >33, it may indicate that the detected specimen contains lower concentration of cells, extracted nucleic acid was degraded or certain inhibitors were present in the reaction.
2. In the Ct value of HEX/VIC channel is ≤33, analyse the results according to the following table:

Table 6 – Interpretation of the results

Interpreting test results		**FAM (ORF1ab gene)**	
		Ct ≤ 33	**No Ct or Ct > 33**
Cy5 (N gene)	**Ct ≤ 33**	SARS-CoV2 ***POSITIVE***	Test again and if repeated is ***NEGATIVE***. If not: ***SUSPICIOUS***
	No Ct or Ct > 33	Test again and if repeated is ***POSITIVE***. If not: ***SUSPICIOUS***	SARS-CoV2 ***NEGATIVE***

No requirement for HEX/VIC channel test results, if the sample is extracted from virus culture.

For SUSPICIOUS samples, it is recommended to re-collect the specimen or change the collection location, then test the specimen again.

7. PERFORMANCES

7.1. Limit of detection: 200 copies/mL

7.2. Precision: using precision reference CV1 and CV2 for within-batch and between-batch detection, the coefficient of variatin (CV) of their Ct values is **<5.0%.**

7.3. Conformity Rate of negative control: 100%

7.4. Conformity Rate of positive control: 100%

7.5. Specificity

Non-specific interference of influenza A Virus (H1N1, H3N2, H7N9, H5N1), Influenza B Virus (Yamagata, Victoria), RSV (Type B), Respiratory Adenovirus (Type 3, Type 7), Haemophilus influenzae, Staphilococcus aureus, Streptococcus pneumoniae, etc has been excluded in repeated experiments.

7.6 Assay turnaround time

The test takes about 2 hours from amplification to results, plus additional 30 – 60 min for the RNA extraction test, depending on the extraction method in use.

8. TROUBLESHOOTING

Problem 1: lack of signal

Possible causes:

1. Wrong channel/filter.
2. Pipetting error: a reagent or a sample was omitted.
3. Inhibitors effect: total RNA with inadequate purification and/or extraction.

Solutions:

- Check the correct setup of the thermalcycler and reading channel thermal profile.
- Repeat the experiment using the same RNA sample.
- Repeat the experiment using RNA extracted in a new DNA extraction run.

Problem 2: low fluorescence intensity

Possible causes:

1. Fluorophore deterioration and/or primers degradation due to inappropriate kit storage.
2. Insufficient or excessive RNA quantity and/or inadequately pure RNA.

Solutions:

- Check the storage of the kit
- Optimize the steps and the timing of the pre-analytical step of total RNA extraction.

Problem 3: variable fluorescence intensity

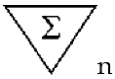
Possible causes:

1. The TOTAL MIX was not mixed properly
2. Presence of air bubbles in the PCR tubes

Solutions:

- Mix carefully the TOTAL MIX in a homogeneous way and spin briefly
- Pipette the TOTAL MIX and the sample in the tube, avoiding the formation of air bubbles. If possible, centrifuge briefly the plate/tube

9. SYMBOLS

	**Manufacturer** **Fabbricante**		**For in vitro diagnostic use only** **Per solo uso diagnostico in vitro**
	**Authorized representative** **Rappresentante autorizzato**		**Consult instructions for use** **Leggere le istruzioni per uso**
	**Contains sufficient for <n> tests** **Contiene material per <n> test**		**Keep dry** **Mantenere all'asciutto**
	**Catalogue code** **Codice di catalogo**		**Temperature limitations** **Limiti di temperature**
	**Lot number** **Numero del lotto**		**Use by** **Utilizzare entro il**
	**Compliant to 98/79/EC directive** **Rispetta la direttiva 98/79/EC**		**Use only once** **Usare solo una volta**

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