Instructions for Use

Test Kit for SARS-CoV-2 RNA Detection in Biological Material Using PCR Method

REF. No. LPC-C-100IC

For Use with LifePad Analyzer

Revision 2.0.

2021

K.K. Mirai Genomics

Ask Sanshin Building 3F, 2-6-29, Tsurumi-Chuo, Tsurumi-ku, Yokohama, Kanagawa, Japan

Postal code: 230-0051

Phone: +81 45 510 0607

email: miraigenomics@miraigenomics.com

LIFEPAD EUROPE UNIPESSOAL LDA

Rua Manuel Pinheiro Chagas 9, 4A, Oeiras 2780-069, Portugal

PROPRIETARY NAME

Test Kit for SARS-CoV-2 RNA Detection in Biological Material Using PCR Method

1. INTENDED USE

Test Kit for SARS-CoV-2 RNA Detection in Biological Material Using PCR Method (the Kit) is intended to perform the SARS-CoV-2 viral RNA extraction in nasopharyngeal, oropharyngeal, or combined oro- and nasopharyngeal swab collected from individuals who are suspected of COVID-19 infection, master mix preparation and further SARS-CoV-2 viral RNA detection in the specimen using PCR method.

Results are used for the SARS-CoV-2 RNA identification. Positive results are indicative of the SARS-CoV-2 RNA presence. To determine a patient's infection status, clinical comparability with patient clinical history and other diagnostic information is required. Negative results do not preclude the SARS-CoV-2 infection and should not be used as the sole basis for treatment. Negative results must be combined with clinical observations, patient clinical history, and epidemiological information. The Kit is intended to be performed by trained users in both laboratory and near patient testing settings.

The Kit is intended for use with the LifePad Analyzer (the Analyzer).

2. SUMMARY AND EXPLANATION

Originated at Wuhan, Hubei province, China in early December 2019, coronavirus disease 2019 (COVID-19) has rapidly widespread worldwide and has led to ongoing global pandemic declared by World Health Organization (WHO) on March 11, 2020. The etiological agent of COVID-19 was designated as **severe acute respiratory syndrome coronavirus 2** (SARS-CoV-2, previously referred to as the 2019 novel coronavirus (2019-nCoV) as well). It is believed to have zoonotic origins and has close genetic similarity to bat coronaviruses, suggesting it emerged from a bat-borne virus. Human transmission primarily occurs through direct, indirect, or close contact with infected people through infected secretions such as respiratory secretions, saliva or through respiratory droplets that are expelled when an infected person coughs, sneezes, or speaks. The mortality of COVID-19 patients is increasing logarithmically and is mostly observed in older age people and patients having history of chronic ailments like chronic obstructive pulmonary disease (COPD), hypertension, diabetes, cardiovascular & cerebrovascular dysfunction, compromised immunity, renal comorbidities, hepatic, obesity problems etc., and recently investigated thrombotic complications

The disease is mainly diagnosed by the viral RNA detection in nasopharyngeal swabs by polymerase chain reaction.

Test Kit for SARS-CoV-2 RNA Detection in Biological Material Using PCR Method is an *in vitro* diagnostic test based on the nucleic acid amplification. It specifically identifies the SARS-CoV-2 RNA sequences that comprise the genetic material of the virus.

3. PROCEDURE PRINCIPLE

The extraction method is based on the selective adsorption of the nucleic acids to the column filter material. The extraction of the SARS-CoV-2 RNA from nasopharyngeal, oropharyngeal, and combined oro- and nasopharyngeal swabs using the Kit and the Analyzer includes 5 consecutive stages and consists of:

1) Virus inactivation and 2) lysing of the specimen in the SSB (swab suspension buffer) solution
3) Collecting the RNA on the filter column.
4) Purifying and 5) collecting the eluate containing purified target RNA.

The resulting specimen is then used for PCR.

The stages of the RNA extraction using the Kit and the Analyzer are as follows:

- During the first stage of the sample preparation, the specimen and Internal Positive Control (IPC) are suspended in the SSB lysis buffer. The lysis buffer is composed of ethanol and a chaotropic agent (guanidine thiocyanate) giving the buffer its protein denaturation properties and allowing effective viral particle lysis.
  As a result, viral capsid proteins lose their structure, viral particles are disintegrated, and viral genetic material (a single-stranded RNA that includes highly conserved sequence targeted for the detection) is released into solution.
- During the second stage, the SSB lysis buffer, that already contains lysed viral particles, and IPC pass through a filter. The viral RNA is retained in the filter, while remaining specimens together with the SSB lysis buffer that could interfere with the reaction pass through the filter.
- Two successive washing stages are performed to ensure complete removal of all interfering substances using the WS1 washing solution and the ethanol-based WS2 washing solution.
- During the next stage, the filter membrane has to be dried completely to ensure no ethanol residue remains. Ethyl alcohol may interfere with the reaction by inhibiting nucleic acid amplification. During both washing and drying stages, the target RNA and IPC are retained with the filter.
- During the fifth stage, RNase free ultra-pure water (EB solution) passes through the filter to elute the target RNA and IPC.

The stages of the master mix preparation are as follows:

- At the sixth stage, the eluate is automatically dosed and mixed with reagent E (Enzymes) and IPC.
- At the seventh stage, automatic dosing and mixing with reagent P (Primers) takes place.

The method is based on the detection of specific viral RNA fragments (Nsp15, N) by obtaining a DNA copy (cDNA) from the RNA matrix using a reverse transcription reaction and its accumulation (amplification) using PCR method.

The amplification is carried out by Aac pol I, a DNA polymerase modified enzyme, derived from *Alicyclobacillus acidocaldarius*, a thermophilic bacterium, using a set of asymmetric primers at a constant temperature. The reaction consists of 2 stages. At the first stage, a linear single-stranded cDNA molecule containing the target amplified site is formed. At the second stage, a non-cyclic amplification of the target fragment occurs with the formation of a set of concatemers (DNA molecules containing several copies of the target DNA). Due to the large number of primers, the reaction has a high specificity, sensitivity and speed.
Fluorescence detection using an exciton primer labeled with two thiazole orange molecules allows the registration of amplification products during the reaction ("in real time"). The detection of amplification products is performed in a reaction solution. During amplification, the exciton primer binds to the synthesized concatemer chain, while the fluorescent dye molecules associated with it intercalate into the formed double-stranded DNA-DNA hybrid, which leads to an increase in fluorescence and makes it possible to register it on a fluorescent detector.
The amplification products registration during the reaction ("in real time") performed with a certain periodicity (in each "cycle" of amplification). Using specially selected settings, the analyzing program automatically calculates the cycles of intersection of the accumulation curves of the fluorescent signal with the threshold line (Cycle threshold, Ct).

4. REAGENTS AND INSTRUMENTS

4.1 Materials Provided

The Kit contains sufficient materials and reagents to process 100 specimens and 2 quality control samples.

1. Test Cartridge for SARS-CoV-2 Detection Using PCR Method, in individual packaging **102 pcs**

2. Tube with the SSB solution for the biological sample dilution **100 tubes**

2.1 SSB solution, clear colorless liquid, 1 mL

3. Cap with dropper nozzle **100 pcs**

4. Positive control sample:

4.1 SSB solution, clear colorless liquid, 1 mL **1 tube**
4.2 Cap with dropper nozzle **2 pcs**
4.3 Positive control sample **1 tube**

5. Negative control sample:

5.1 SSB solution, clear colorless liquid, 1 mL **1 tube**
5.2 Cap with dropper nozzle **1 pc**

6. Operational documents:

6.1 Instructions for Use **1 pc**
6.2 Datasheet **1 pc**

7. Disposable sterile swab, individually packed **100 pcs**

4.1.1. COMPOSITION OF REAGENTS

The composition of the reagents used in the Kit is shown in Table 1.

Table 1. Composition of Reagents

Name of the component	Composition	The final concentration in the reagent
SSB Solution	Guanidine Thiocyanate	2.4 M
	Sodium polyacrylate	0.0025%
	Tris-HCl buffer solution, pH 7.4	25 mmol
	Triton X-100	0.5%
	Ethanol	33%
Test Cartridge		
WS1 Solution	Guanidine hydrochloride	2.4 M
	Ethanol	50%
WS2 Solution	Ethanol	95%
EB Solution	Nuclease-free distilled water	100%
Reagent E	Trisma Potassium Acetate Ammonium Sulfate Magnesium Sulfate Twin 20 dNTP Non-nuclease distilled water	---

	AacPoll DNA Polymerase AMV-RT Revertase	
Reagent P	Nuclease-free distilled water 6 asymmetric SmartAmp Primers	---
Internal Positive Control (IPC)	Dry mixture of an in vitro synthesized fragment of the RuBisCO RNA genome	$4x10^5$ copies/mL
Positive Control Sample	Dry mixture of an in vitro synthesized fragment of the SARS-CoV-2 RNA genome	$4x10^7$ copies/mL

4.2 Materials Required but Not Provided

- LifePad Analyzer.
- PC (varies by configuration of the Analyzer, could be included to the scope of supply).
- Barcode scanner (varies by configuration of the Analyzer, could be included to the scope of supply).

5. STORAGE AND HANDLING

- The Kit shelf life is 12 months.
- Do not use the Kit after its expiry date.
- Stability of the kit components after the package opening is within the period of 12 months, subject to storage conditions.
- Store the kits at +2°C to +30°C.
- Avoid exposure to direct sunlight, high atmospheric humidity, and an aggressive environment.
- Do not freeze.
- Do not open a cartridge lid until you are ready to perform testing.

6. WARNING AND PRECAUTIONS

6.1 General

- For *in vitro* diagnostic use.
- This product has been authorized only for the detection of the SARS-CoV-2 nucleic acid, not for any other virus or pathogens.
- Positive results are indicative of the SARS-CoV-2 RNA presence.
- Laboratories within the European Union are required to report all results to the appropriate public health authorities.

NOTE: All biological specimens including used cartridges should be handled using standard precautions and treated as if capable of transmitting infectious agents.

- Follow safety procedures set by your institution and local authorities for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material. Check relevant state and local

regulations.

6.2 Specimens

- Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen (see Section 9, Specimen Collection, Transport, and Storage). Specimen stability under shipping conditions other than those recommended has not been evaluated.

6.3 Assay/Reagent

- Wear clean lab coats and gloves. Change gloves between the handling of each specimen.
- Do not open the Test Cartridge lid except when adding a specimen.
- Each Test Cartridge is single-use and is intended to process one test. Do not reuse the previously used cartridges.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not use a cartridge with a damaged protective film.
- Do not use a damaged SSB tube.
- Do not use a cartridge if it appears wet.
- In the event of specimens/controls spill, wear gloves and mask and absorb the spill with paper towels. Clean the contaminated area with a 10% chlorine bleach. Minimal exposure time is 3 minutes. Ensure the work area is dry before using 70% denatured ethanol to remove the bleach residue. Allow the surface to dry completely before starting the work.
- Biological specimens, transfer devices, and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow local environmental waste procedures for proper disposal of used cartridges and unused reagents.

7. CHEMICAL HAZARDS

Signal Word: Warning

UN GHS HAZARD STATEMENTS

- Harmful if swallowed.
- May be harmful in contact with skin.
- Causes eye irritation.

PRECAUTIONARY STATEMENTS

Prevention

- Wash hands thoroughly after handling.
- Do not drink, eat, or smoke when using the product.

Response

- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- If eye irritation persists: Seek for medical advice.
- IF ON SKIN: Wash with plenty of soap and water.
- If skin irritation occurs: Seek for medical advice.
- IF SWALLOWED: Rinse mouth. Call a POISON CENTER or doctor/physician if you feel unwell.

8. SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

NOTE: Proper specimen collection, storage, and transport are critical to the test performance. Inadequate specimen collection, improper specimen handling and/or transport may lead to a false result.

The Kit is intended to perform the SARS-CoV-2 detection in nasopharyngeal or oropharyngeal swabs.

Each specimen should be immediately placed in a separate tube with the SSB solution included in the kit at the site of collection.

Treat all biological specimens as if capable of transmitting infectious agents. Refer to the WHO Laboratory biosafety guidance related to coronavirus disease (COVID-19) for details (https://www.who.int/publications-detail/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)).

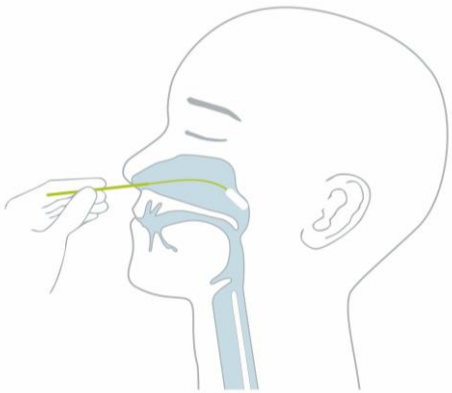
Specimen storage and transportation conditions are:

- At room temperature — within 24 hours.
- At temperatures from +2 to +8°C — within 7 days.
- At a temperature of -20°C — within 1 month.

8.1 Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Picture 1) until you feel a slight resistance. Rotate the swab by firmly brushing against the nasopharynx for 5 seconds.

Place the swab into the tube with the SSB solution and suspend it. Dispose of the swab according to the local clinical waste disposal regulations.



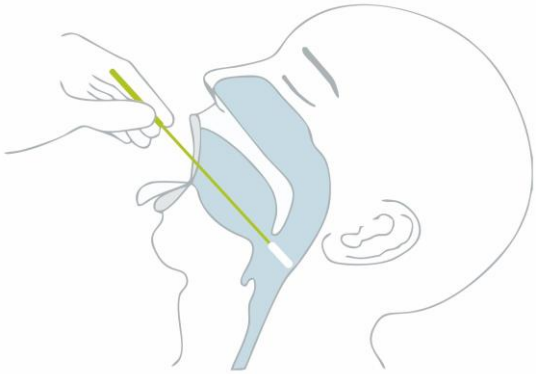
Picture 1. Nasopharyngeal Swab Collection

NOTE: The resulting lysate does not contain any viable viruses and can be used for the testing procedure.

8.2 Oropharyngeal Swab Collection Procedure

Ask the patient to say "ah-ah" to raise the uvula. Press down on the tongue with a spatula. Smoothly take a smear from the back of the throat and the arch of the tonsils (see Picture 2). Avoid swabbing the soft palate or touching the tongue with the swab tip.

Place the swab into the tube with the SSB solution and suspend it. Dispose of the swab according to the local clinical waste disposal regulations.



Picture 2. Oropharyngeal Swab Collection

NOTE: The resulting lysate does not contain any viable viruses and can be used for the testing procedure.

8.3 Combined Oro-Nasopharyngeal Swab Collection Procedure

Insert the swab into either nostril, passing it into the posterior nasopharynx (see Picture 1) until you feel a slight resistance. Rotate the swab by firmly brushing against the nasopharynx for 5 seconds. Repeat for the second nostril with the same swab. Place the swab into the tube with the SSB solution.

Take a new swab. Ask the patient to say "ah-ah" to raise the uvula. Press down on the tongue with a spatula. Smoothly take a smear from the back of the throat and the arch of the tonsils (see Picture 2). Avoid swabbing the soft palate or touching the tongue with the swab tip.

Place the swab into the same tube with the SSB solution. Suspend both swabs in the tube and dispose of them according to the local clinical waste disposal regulations.

NOTE: The resulting lysate does not contain any viable viruses and can be used for the testing procedure.

9. TESTING PROCEDURE

The consumption of **the Kit components** per one detection is indicated in the table below:

Table 2. The Kit Consumption Per One (1) Detection Procedure

Component	Function	Required volume per sample
Tube with the SSB solution for the biological sample dilution	Lysis buffer	1.0 mL
Test Cartridge for SARS-CoV-2 Detection Using PCR Method, in individual foil packaging	SARS-CoV-2 detection	1 pc.
Disposable sterile swab, individually packed	Specimen collection	1 pc.

9.1 REAGENTS PREPARATION AND EXTRACTION PROCEDURE

9.1.1. Preparing for testing

I. Switch on the LifePad Analyzer as described in the User Manual.
II. Take the Test Cartridge out of the package and check for any damages.

NOTE: Do not use the Kit components with damaged sealed packaging.
Do not use the Test Cartridge with visual defects, solutions external spreading over the surfaces, or internal spreading through channels.
Inform the manufacturer in case any signs of the solution spreading are present (see Section 20, Warranty).

III. Enter the patient's data at the designated place on the Test Cartridge label.
IV. Tear off the protective film from the Test Cartridge.
V. Insert the Test Cartridge into the Analyzer to the indicated dotted line, check if the Test Cartridge installed correctly in the LifePad software as described in the User Manual.
VI. Place the tubes with SSB solution with clinical material onto the working table with the Analyzer to enter the patient ID into the LifePad software as described in the User Manual.

9.1.2. Testing:

I. Place a cap with a dropper nozzle firmly onto the tubes with the SSB solution containing a specimen.
II. Add 500 µL of the solution (see Line I) into the inlet up to the red mark on the Test Cartridge.
III. If the specimen retest is required, use the remaining 500 µL of the solution.
IV. Close the Test Cartridge lid.
V. Shake the Test Cartridge for 3-5 seconds.
VI. Tear off the protective film.
VII. Dispose of the used tubes with the SSB solution according to the local guidelines and

regulations.

VIII. Use the barcode reading function if available in the Analyzer[1]. Enter the patient's data in the ASSAYS window of the LifePad software. The test results are associated with the patient ID.

IX. Start testing in the LifePad software as described in the Analyzer User Manual.

X. Interpret the results as described in the Analyzer User Manual.

NOTE: Use a new sealed Test Cartridge for each new test.
Do not reuse Test Cartridges that have partially undergone the testing with the Analyzer.

10. QUALITY CONTROL

10.1 Internal Controls

IPC ensures that the RNA is extracted correctly and verifies that the specimen processing is adequate.

Please refer to the table below to find out the possible IPC amplification results.

Table 3. Possible IPC Amplification Results

Result	SARS-CoV-2 is detected	SARS-CoV-2 is not detected	SARS-CoV-2 is detected	Invalid*
IPC	Positive	Positive	Negative	Negative
Specimen (COVID-19)	Positive	Negative	Positive	Negative

** The result cannot be interpreted. Please retest.*

10.2 External (Positive and Negative) Controls

NOTE: To check the Kit and the Analyzer functional characteristics correctness, it is recommended to use the positive and negative controls.

I. Take the Positive Control Sample included in the Kit.

II. Place a cap with a dropper nozzle firmly onto the tube with the SSB solution.

III. Add 1 mL of the solution (see Step II) to the Positive Control Sample labeled tube.

IV. Place a cap with a dropper nozzle firmly onto the Positive Control Sample labeled tube.

V. Add 1 mL of the solution (see Step III) into the inlet of the Test Cartridge.

VI. Dispose of the used tubes according to the local guidelines and regulations.

VII. Use the barcode reading function if available in the Analyzer[2]. Enter the patient's data in the ASSAYS window of the LifePad software. The test results are associated with the patient ID.

VIII. Start testing in the LifePad software as described in the Analyzer User Manual.

IX. Interpret the results as described in the Analyzer User Manual.

Use the same procedure for Negative Control Sample (excluding Steps III-IV). Interpret the

[1] The function is optional.

[2] The function is optional.

negative results as described in the LifePad Analyzer User Manual.

11. VIEWING AND PRINTING RESULTS

For detailed instructions on how to view and print the results, please refer to the *LifePad Analyzer* User Manual.

12. RESULTS INTERPRETATION

The Analyzer automatically interprets the test results and shows them in the software. The Kit provides test results based on the detection of the target RNA according to the algorithms shown in the table below.

Table 4. The Kit Possible Results

RESULT	***COVID***	***IPC***
COVID POSITIVE	**+**	**+/-**
COVID NEGATIVE	**-**	**+**
INVALID	**-**	**-**

Table 5. Results Interpretation

RESULT	**INTERPRETATION**
COVID POSITIVE	The SARS-CoV-2 target RNA is detected. • The result is interpreted based on the fluorescence curse rise presence and the Ct value within the valid range. • IPC: positive/negative; IPC could be ignored since the SARS-CoV-2 target occurred
COVID NEGATIVE	The SARS-CoV-2 target RNA is not detected. • The result is interpreted based on the fluorescence curse rise absence and the Ct value beyond the valid range*. • IPC: positive and has a Ct within the valid range*.
INVALID	IPC doesn't meet the acceptance criteria. Presence or absence of the SARS-CoV-2 target RNA cannot be determined. Repeat test according to the Retest Procedure described in Section 14.1.

**For the Ct valid ranges, please refer to the LifePad Analyzer User Manual*

13. RETEST

13.1 Reasons for Retest

- An *INVALID* result indicates that the IPC testing failed.
- An *ERROR* result could be due to, but not limited to, the Analyzer components failure, or

if the maximum and minimum pressure limits.

13.2 Retest Procedure

To retest an invalid result, use a new cartridge. Use the leftover sample from the original specimen in the tube with the SSB solution.

Repeat Steps I-X described in Section 10.1.2., Testing.

14. POOLING

Pooling (sometimes referred to as the *pool testing*) is combining the same specimen type from several people and performing the combined pool of specimens to detect SARS-CoV-2. It could help save limited COVID-19 testing resources and make the testing process more efficient.

Pooled tests that return positive results require each specimen in the pool to be retested individually prior to reporting a result to determine which individual(s) are positive.

Using the Kit and the LifePad Analyzer, up to five specimens could be tested in a single pool. The pool size depends on the required efficiency gains, the SARS-CoV-2 positivity rate in the tested population, and the potential risks of pooling.

Combination of multiple specimen types in a pool has not been validated.

Note: Specimens with low SARS-CoV-2 RNA concentrations may not be detected in specimen pools due to the decreased sensitivity of pool testing. The laboratories are recommended to consider the risks of decreased test sensitivity when performing pooling and only use pooling if the risks do not outweigh the benefits of saving resources.

- Ensure traceability between individual specimen IDs and pool IDs.
- To reduce any potential contamination of the LifePad Analyzer, use single-use pipette tips when transferring the specimens into secondary tubes.
- Ensure appropriate specimen handling techniques to reduce the risk of cross-contamination of pools and original patient specimens.

14.1 Pooling methods

1. Identify a uniquely labeled secondary tube (pooled specimen tube) for pooling.

2. To avoid confusion, associate the specimens to be pooled with the pool tube, using either a laboratory validated specimens tracking system or pooling worksheets.

3. Work with only the specimens for one pool at a time if performing manual pooling.

4. Ensure each specimen has sufficient volume for pool construction and any possible resolution testing (pool deconvolution) that may be further required. Example: for pools of 5, a minimum volume of 250 µL (for pool) plus 500 µL (for resolution) is required for a minimum specimen volume of 750 µL available prior to pooling (Table 6). Prepare a secondary tube containing the target pool volume for further volume comparison if performing manual pooling.

Table 6. Minimum Specimen Volume for Pooling

Pool Size	Volume Required for Pool (µL)	Volume Required for Resolution Testing (µL)	Minimum Volume Required Prior to Pooling (µL)
5	250	500	750
4	300	500	800
3	400	500	900
2	500	500	1,000

5. To prepare the pool, carefully transfer each individual specimen associated with the pool into the appropriate secondary tube using a calibrated micropipette with a new pipette tip for each specimen. Ensure that pipette tips are filter/barrier tips, clean and lifted directly from pipette-tip racks.

6. Ensure complete mixing after addition of all specimens to the secondary tube (i.e., through pipetting up and down). Avoid creating bubbles, foam or aerosols while mixing.

7. Visually compare the pooled specimen volume in the secondary tube to a secondary tube containing the target pool volume if performing manual pooling. If the pooling tube level is less or more than the standard pool volume, then the manually prepared pool should be discarded and prepared again.

8. Process pooled specimen using the LifePad Analyzer as described in the LifePad Analyzer User Manual.

14.2 Pool Result Interpretation and Reporting

Pool results interpretation is the same as for individual results as described in Section 13, Results Interpretation.

- If the pool result is negative, then each constituent specimen is reported as negative. The result report should include a comment that pooling was used during testing.
- If the pool result is positive, then each of the constituent specimens must be re-tested as a separate individual specimen. Use the laboratory defined tracking system to ensure the correct individual specimens are tested. Individual test results supersede the pool result.
- If the pool result of the is invalid, each constituent specimen should be re-tested as a separate individual specimen in order to avoid potential delay in reporting valid patient results. However, if the invalid is due to entire run failure or other LifePad Analyzer malfunctions, the pool may be re-tested if sufficient volume is available.

15. LIMITATIONS

- The viral RNA may not be detected if the viral concentration is less than $1x10^3$ copies/mL.
- The Kit performance characteristics have been evaluated only for the specimen types described in the Section 2, Intended Use.
- The Kit cannot rule out diseases caused by other bacterial or viral pathogens.
- A false negative result may occur if a specimen is improperly collected, transported or handled.

16. CARRY-OVER CONTAMINATION

To keep any possible test-to-test contamination to a minimum, specimens and solutions that could contain amplicons are contained within the single-use, disposable Test Cartridge. The Test Cartridge design prevents the Analyzer from contacting any solutions within the Cartridge. No manual specimen processing steps are required other than the addition of the specimen to the cartridge before test start. The Test Cartridge and the Analyzer is a closed system since once the specimen is added to the Test Cartridge, the Test Cartridge lid is closed. Thus the instrument and cartridge design are a closed system which minimizes the potential for carry-over.

17. PERFORMANCE CHARACTERISTICS

17.1. Clinical Evaluation

The performance of the Kit was evaluated using specimens taken from patients during the treatment and diagnostic process. The diagnostic sensitivity of the SARS-CoV-2 RNA detection was evaluated using 200 positive specimens (100 nasopharyngeal swabs and 100 oropharyngeal swabs containing the SARS-CoV-2 RNA) with a confidence interval (CI) equal to 95%. The diagnostic specificity of the SARS-CoV-2 RNA detection was evaluated using 200 negative specimens (100 nasopharyngeal swabs and 100 oropharyngeal swabs that do not contain the SARS-CoV-2 RNA) with a confidence interval of 95%.

Totally tested:	
True-positives:	**200**
False-positives:	**0**
True-negatives:	**200**
False-negatives:	**4**

Diagnostic sensitivity: 98.04% (95% CI 95.06-99.46%).

Diagnostic specificity: 100.00% (95% CI: 98.17-100.00%).

Diagnostic sensitivity when testing the nasopharyngeal swabs is 98.04% (95% CI 93.10-99.76%).

Diagnostic specificity when testing the nasopharyngeal swabs is 100,00% (95% CI 96,38-100,00%).

Diagnostic sensitivity when testing the oropharyngeal swabs is 98.04% (95% CI 93.10-99.76%).

Diagnostic specificity when testing the oropharyngeal swabs 100.00% (95% CI 96.38-100.00%).

18. ANALYTICAL PERFORMANCE

18.1 Limit of Detection (Analytical Sensitivity)

Studies were performed to determine the analytical limit of detection (LoD) of the Kit.

Table 8. Limit of Detection

SARS-CoV-2 Concentration in the Specimen	Results	
	Number of positive results / number of tests	% of positive results

2 × LoD (2×10^3 copies/mL)	10 / 10	100
1 × LoD (1×10^3 copies/mL)	20 / 20	100
0.5 × LoD (5×10^2 copies/mL)	6 / 10	60

The claimed LoD is 1×10^3 copies/mL.

18.2 Analytical Specificity (Exclusivity)

Study of possible cross-reactions with all the organisms listed in Table 9 was performed.

The cross-reaction with the pathogens used was not detected.

Table 9. Bacteria and Viruses Used for the Cross-reactivity Study

No.	Causative Agent	No.	Causative Agent	No.	Causative Agent
P1	Human respiratory syncytial virus, hRSV-1 strain	P8	Human Metapneumovirus – HM-1	P15	Influenza A H3N2
P2	Streptococcus pneumoniae ATCC® 49619™	P9	Human Adenovirus (B, C, E)	P16	Human Parainfluenza virus 2/4
P3	Haemophilus influenzae ATCC® 49247™	P10	Human Parainfluenza virus 1/3	P17	Human Coronavirus HKU1 / Human Coronavirus OC43
P4	Human parainfluenza virus 3, Bok	P11	Bocaparvovirus	P18	Human Coronavirus NL63 / Human Coronavirus 229E
P5	Influenza A A/Anhui/1/2013 (H7N9)	P12	Staphylococcus aureus	P19	Human Rhinovirus
P6	Adenovirus 5 394	P13	Human Metapneumovirus		-
P7	Influenza B B/Moscow/46/2019	P14	Human respiratory syncytial virus	-	-

Table 10. Cross-reactivity Assessment

Sample No.	False-positive results / number of tests	Specimen No.	False-positive results / number of tests
P1	0/5	P11	0/5
P2	0/5	P12	0/5
P3	0/5	P13	0/5
P4	0/5	P14	0/5
P5	0/5	P15	0/5
P6	0/5	P16	0/5
P7	0/5	P17	0/5
P8	0/5	P18	0/5
P9	0/5	P19	0/5
P10	0/5	-	-

18.3 Analytical Reactivity (Inclusivity)

The inclusivity of the Kit was evaluated by *in silico* analysis in relation to 6,037,339 SARS-CoV-2 sequences available (as of December 13, 2021) in the database of National Genomics Data Center (NGDC) of the China National Center for Bioinformation (CNCB) for two targets, Nsp15 and N-region.

For the sequences of Nsp15, 98.5% (138/140 bases) sequences had a low mutation ratio (<0.1%) except for two bases. Each mutation is located at position 19,839 and 19,859 on the reference genome of SARS-CoV-2 (NC_044512.2). The detailed information of each mutation is shown as follows:

- The mutation ratio at position 19,839 was 0.63%. The major variant at the position is SNP-like point mutation (T>C). Since the mutation is located at the 15th base from 3'-end of Turnback primer (TP), there is no severe effect for the priming.
- The mutation ratio at position 19,859 was 0.21%, it was not such a high mutation rate. The major variant at the position is SNP-like point mutation (C>T). The mutation is located at the 2nd base from the 3'-end of internal boost primer (iBP).

$$\mathrm{VIS}_x = \frac{\text{Increasing rate of the numbers of variant at position } x \text{ on SARS-CoV-2 genome among certain period}}{\text{Increasing rate of total numbers of the genome among certain period}}$$

For the sequences of N-region, 96.7% (120/124 bases) sequences had a low mutation ratio (<0.1%) except for 4 bases. The detailed information of each mutation is shown as follows:

- The mutation ratio at position 28,869 was 1.82%. The major variant at the position is SNP-like point mutation (C>T). Since the mutation is located at the 7th base from the 3'-end of TP-turnback sequence, there is no severe effect for the amplification.
- The mutation ratio at position 28,932 was 2.04%. The major variant at the position is SNP-like point mutation (C>T). Since the mutation is located at the 13th base from 3'-end of the Outer boost primer (oBP), there is no severe effect for the priming.
- The mutation ratio at position 28,975 was 1.76%. The major variant at the position is SNP-like point mutation (G>T, G>A or G>C). Since the mutation is located at the 5th base from 3'-end of Outer primer (OP), there is no severe effect for the priming.
- The mutation ratio at position 28,977 was 13.25%. The major variant at the position is SNP-like point mutation (C>T). Although the mutation is also located at OP, the position is the 3rd base from 3'-end of the primer. The mutation ratio is quite high, however 98.0% (783,882/800,067) of the mutation was shown on the genome of B.1.1.7 linage, which is 'α' strain. Therefore, the mutation is not at high risk of the positive results from recent epidemic situations.

Additionally, Table 11 shows the transition of each mutation ratio from 4 months ago (August 25, 2021) to now (December 13, 2021). Right column has a value of variant increasing score (VIS), which is calculated by following a formula:

Here, except for a mutation at position 19,859 of SARS-CoV-2 genome, the expansion rate of each mutation decreased, and each VIS was about 0.3, respectively. This is suggested that each mutation has low possibility as the future candidate of critical mutation even if the current high frequency of the mutation is position 28,977 (13.25%). On the other hand, the VIS of a mutation at position 19,859 on Nsp15 was 3.03, it may become a major mutation of SARS-CoV-2 in the future. However, the current mutation ratio is still low (0.021%) and this mutation position is located at iBP. Different from TP, Folding primer (FP) and Epimer having a major role in the Kit, the iBP is one of supportive primers to accelerate the reaction. Therefore, it does not contribute to the results of the amplification.

Table 11. Transition of Each Mutation Frequency from August 25, 2021 to December 13, 2021

(1) High-frequent valiants (>0.1% at 13 Dec. 2021) on Nsp15-primer sequences

Primer name	SARS-CoV-2 Genomic position (Reference genome, NC_045512.2)	Position from 3'-end of each primer	1,797,460 sequences at 25 Aug. 2021		6,037,339 sequences at 13 Dec. 2021		VIS (Variant increasing score)
			Number of mutation	Frequency	Number of mutation	Frequency	
TP (anneal)	19839	15	35728	1.99%	38095	0.63%	0.32
iBP	19859	2	1258	0.07%	12802	0.21%	3.03

(2) High-frequent valiants (>0.1% at 13 Dec. 2021) at N region-primer sequences

Primer name	SARS-CoV-2 Genomic position (Reference genome, NC_045512.2)	Position from 3'-end of each primer	1,797,460 sequences at 25 Aug. 2021		6,037,339 sequences at 13 Dec. 2021		VIS (Variant increasing score)
			Number of mutation	Frequency	Number of mutation	Frequency	
TP (turn-back)	28869	7	102765	5.70%	109605	1.82%	0.32
oBP	28932	13	121208	6.70%	123260	2.04%	0.30
OP(f)	28975	5	97882	5.40%	106413	1.76%	0.32
	28977	3	794343	44.20%	800,067	13.25%	0.30

Therefore, the Kit was predicted to have a low possibility of unavailability against the mutated SARS-CoV-2 strain.

18.4 Interfering Substances

To study the possible effect of interfering substances, potential interferents (hemoglobin (human blood), mucin (saliva), chlorhexidine bigluconate) were added to positive specimens.

Table 12. Interference Study Results

Specimen Type	Number of specimens tested	Interfering Substance	Interfering Substance Concentration	Interference
Nasopharyngeal swab	6	Mucin	5% (v/v)	Not detected
	6	Hemoglobin	2.5% (v/v)	Not detected
	6	Chlorhexidine bigluconate aqueous solution	2% (v/v)	Not detected
Oropharyngeal swab	6	Mucin	5% (v/v)	Not detected
	6	Hemoglobin	2.5% (v/v)	Not detected
	6	Chlorhexidine bigluconate aqueous solution	2% (v/v)	Not detected

No false-positive results were detected as well when adding the above interferents into negative specimens.

19. REPRODUCIBILITY

To verify reproducibility, the GK2020/1 SARS-CoV-2 strain with a concentration equal to 2 × LoD, LoD 0.5 × LoD, as well as 3 positive and 2 negative clinical specimens were tested using the Kit.

The coefficient of variation (CV) does not exceed claimed 20% when assessing reproducibility testing with one Analyzer, two Analyzers and the cluster of Analyzers, including when using different Kit lots.

20. WARRANTY

The manufacturer guarantees the compliance of the Kit functional characteristics with the requirements specified in the technical and operational documentation, within the established shelf life (12 months), subject to all conditions of transportation, storage and use.

21. TECHNICAL ASSISTANCE

If any technical assistance is required, please contact us at:

Phone: +81 45 510 0607

email: miraigenomics@miraigenomics.com

Table of Symbols

	Catalog number
	Manufacturer
	Production date
	Expiry date
	Lot
	*In vitro* diagnostic medical device
 **100**	The Kit content is enough to run 100 tests
	Do not reuse
	Warning! Consult the Instructions for Use
	Consult the Instructions for Use
	Do not use if the package is damaged
	Storage temperature limitations
	Keep dry
	Non-sterile