RIDA®GENE SARS-CoV-2 RUO

REF PG6815RUO

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English

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1. Intended use

For research use only. Not intended for diagnostic procedures.
RIDA®GENE SARS-CoV-2 RUO test, which will be performed on real-time PCR instruments (RIDA®CYCLER, LightCycler®480II, Mx3005P; ABI 7500, CFX96™ and Rotor-Gene Q), is a multiplex real-time RT-PCR for the direct qualitative detection of novel coronavirus (SARS-CoV-2) RNA from human respiratory samples.
The product is intended for use by professional users in hospital laboratories, reference laboratories, private laboratories or state laboratories.

2. Summary and explanation of the test

At the end of December in the Chinese metropolis of Wuhan, numerous cases of pneumonia of unknown cause occurred.[1] At the beginning of January, Chinese authorities identified a new type of corona virus (SARS-CoV-2) as the cause.[1] The disease caused by SARS-CoV-2 is officially named COVID-19 („Corona Virus disease 2019“) and is transmissible from person to person.[2] Worldwide, 45,171 cases have been reported to date (as of February 12, 2020).[3] The initial cases in Germany were confirmed at the end of January 2020.[4]
The WHO declared an international health emergency on January 31, 2020.[1,4]

3. Test principle

RIDA®GENE SARS-CoV-2 RUO is a multiplex real-time RT-PCR for the direct qualitative detection of novel coronavirus (SARS-CoV-2) RNA from human respiratory samples.
Detection is done in a one-step real-time RT-PCR format: reverse transcription (RT) and subsequent PCR take place in one reaction vial. In the process, the isolated RNA is transcribed into cDNA with the help of a reverse transcriptase. The specific gene fragments for SARS-CoV-2 (E gene) are then amplified using real-time PCR. The amplified target sequences are detected using hydrolysis probes that are labeled at one end with a quencher and a fluorescent reporter dye (fluorophore) at the other. The probes hybridize to the amplicon in the presence of a target sequence. During extension, the Taq-Polymerase separates the reporter from the quencher. The

reporter emits a fluorescent signal that is detected by the optical unit of a real-time PCR device. The fluorescent signal increases with the quantity of formed amplicons. The RIDA®GENE Novel Coronavirus 2019 RUO test contains an Internal Control RNA (ICR) to be able to control sample preparation and/or any potential PCR inhibition.

4. Reagents provided

Table 1: Reagents provided (The reagents provided in the kit are sufficient for 100 determinations.)

Kit code	Reagent	Amount		Lid color
1	Reaction Mix	2x	1050 µl	yellow
2	Enzyme Mix	1x	80 µl	red
R	Internal Control RNA	2x	1700 µl	brown
N	No Template Control	1x	450 µl	white
P	Positive Control	1x	200 µl	blue

5. Storage instructions

- All reagents must be stored away from light at -20 °C and, if unopened, can be used until the expiration date printed on the label. After the expiration date, the quality guarantee is no longer valid.
- All reagents should be carefully thawed prior to use (e.g., in a refrigerator at 2 °C - 8 °C).
- Repeated freezing/thawing of up to 5 times does not have an impact on the test property (if necessary, create aliquots after the first thaw and refreeze reagents immediately).
- Cool all reagents appropriately during PCR preparation (2 °C - 8 °C).

6. Additional necessary reagents and necessary equipment

The RIDA®GENE SARS-CoV-2 RUO multiplex real-time RT-PCR test can be used with the following extraction platforms and real-time PCR devices:

Table 2: Necessary equipment

Extraction platforms	
R-Biopharm	RIDA®Xtract
Promega	Maxwell®RSC
Real-time PCR devices	
R-Biopharm	RIDA®CYCLER
Roche	LightCycler®480II
Agilent Technologies	Mx3005P
Applied Biosystems	ABI 7500
Bio-Rad	CFX96™
QIAGEN	Rotor-Gene Q

Note: When using Rotor-Gene Q (QIAGEN), use only 0.1 ml tubes.

Should you have to use other extraction procedures or real-time PCR instruments, please contact R-Biopharm to check the compatibility at mdx@r-biopharm.de.

- RIDA®GENE Color Compensation Kit IV (PG0004) when using LightCycler® 480II
- Real-time PCR consumables (plates, tubes, foil)
- Centrifuge with rotor for reaction vials or plates
- Vortexer
- Pipettes (0.5 - 20 µl, 20 - 200 µl, 100 - 1,000 µl)
- Pipette tips with filters
- Powder-free disposable gloves
- PCR water (nuclease-free)

7. Precautions for users

For research use only.
- This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed.
- Always adhere strictly to the user instructions for carrying out this test.
- Do not pipette samples or reagents using your mouth. Avoid contact with broken skin and mucous membranes.
- Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test.
- Do not smoke, eat, or drink in areas where samples are handled.
- Ensure that the extraction, PCR preparation, and PCR are carried out in different rooms in order to avoid cross-contaminations.
- Clinical samples must be viewed as potentially infectious and must be disposed of appropriately, like all reagents and materials that come into contact with potentially infectious samples.
- Dispose of test kit once the expiration date has lapsed.
- Users are responsible for proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

For further details, see the safety data sheets (SDSs) at www.r-biopharm.com.

8. Collection and storage of samples

8.1 RNA preparation from human respiratory samples

A commercially available nucleic acid extraction kit (e.g., RIDA®Xtract (R-Biopharm)) or nucleic acid extraction system (e.g., Maxwell®RSC (Promega)) is recommended for RNA preparation from human respiratory samples. The manufacturer's instructions must be observed.

The RIDA®GENE Novel Coronavirus 2019 RUO test contains an Internal Control RNA that indicates potential PCR inhibition, checks the integrity of the reagents, and confirms successful nucleic acid extraction. The Internal Control RNA can be used either only as an inhibition control or as an extraction control for sample preparation and as an inhibition control.

If the Internal Control RNA is used only as an inhibition control, 1 µl of the Internal Control RNA must be added to the master mix for each reaction (see Table 4).

If the Internal Control RNA is used as an extraction control for sample preparation **and** as an inhibition control, 20 µl of the Internal Control RNA must be used for each sample during extraction. The Internal Control RNA should be added to the sample/lysis buffer mix and should **not** be added directly to the sample material.

We recommend adding 1 µl for each reaction of the Internal Control RNA to the PCR mix of the negative control and the positive control.

9. Test procedure

9.1 Master Mix preparation

The total number of the reactions needed for PCR (samples and control reactions) must be calculated. One positive control and one negative control must be included in each test run.

Adding an additional 10 % volume to the master mix is recommended in order to balance out the pipette loss (see Table 3, Table 4). Before using the Reaction Mix, thaw the Enzyme Mix, Positive Control, No Template Control, and Internal Control RNA, mix thoroughly and centrifuge for a short time. Always cool reagents appropriately during work steps (2 °C - 8 °C).

Table 3: Example of the calculation and preparation of the master mix for 10 reactions (ICR as extraction and inhibition control)

Kit code	Components of the master mix	Quantity per reaction	10 reactions (plus 10 %)
1	Reaction Mix	19.3 µl	212.3 µl
2	Enzyme Mix	0.7 µl	7.7 µl
	Total	**20 µl**	**220 µl**

Mix the master mix and then centrifuge for short time.

Table 4: Example of the calculation and production of the master mix for ten (10) reactions (ICR only as inhibition control)

Kit code	Components of the master mix	Quantity per reaction	10 reactions (plus 10 %)
1	Reaction Mix	19.3 µl	212.3 µl
2	Taq-Polymerase	0.7 µl	7.7 µl
R	Internal Control RNA	1.0 µl	11 µl
	Total	**21.0 µl**	**231.0 µl**

Mix the master mix and then centrifuge for short time.

9.2 Preparation of the PCR Mix

Pipette 20 µl of the master mix into each reaction vial (vial/plate).

Negative control: Pipette 5 µl of the No Template Control into the pre-pipetted master mix.

Note: **If the Internal Control RNA is used as an extraction control for sample preparation and as an inhibition control, we recommend adding 1 µl of the Internal Control RNA to the RT-PCR mix of the negative control.**

Samples: Add 5 µl eluate to the pre-pipetted master mix.

Positive control: Add 5 µl of the Positive Control to the pre-pipetted master mix.

Note: **If the Internal Control RNA is used as an extraction control for sample preparation and as an inhibition control, we recommend adding 1 µl of the Internal Control RNA to the RT-PCR mix of the positive control.**

Seal the reaction vials or plates, briefly centrifuge at slow speed, and transfer into the real-time PCR device. Start PCR according to PCR instrument set-up (see Table 5, Table 6, Table 7).

9.3 PCR instrument set-up

9.3.1 Universal real-time PCR profile

Table 5: Universal real-time RT-PCR profile for LightCycler® series and RIDA®CYCLER

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation	10 sec, 95 °C
Annealing/Extension	15 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: **Annealing and extension take place in the same step.**

Table 6: Universal real-time RT-PCR profile for Mx3005P, ABI7500, Rotor-Gene Q, and CFX96™

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation	15 sec, 95 °C
Annealing/Extension	30 sec, 60 °C
Temperature Transition Rate / Ramp Rate	Maximum

Note: Annealing and extension take place in the same step.

Note: The universal real-time PCR profile can also be used for DNA tests if RIDA®GENE DNA and RIDA®GENE RNA real-time PCR tests are combined in one run.

9.4 Detection channel setting

Table 7: Selection of appropriate detection channels

Real-time PCR device	Detection	Detection channel	Comment
R-Biopharm RIDA®CYCLER	SARS-CoV-2	Green	**-**
	ICR	Yellow	
Roche LightCycler® 480II	SARS-CoV-2	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
Agilent Technologies Mx3005P	SARS-CoV-2	FAM	**Set the reference dye to none.**
	ICR	HEX	
ABI 7500	SARS-CoV-2	FAM	**Set the ROX passive reference dye to none.**
	ICR	VIC	
Bio-Rad CFX96™	SARS-CoV-2	FAM	**-**
	ICR	VIC	
Qiagen Rotor-Gene Q	SARS-CoV-2	Green	**The gain settings must be set to 5 (factory default) for all channels.**
	ICR	Yellow	

10. Quality control

Samples are evaluated using the analysis software of the respective real-time PCR device according to the manufacturer's instructions. Negative and positive controls must show the correct results (see Table 8).
The Positive Control comes at a concentration of 10^3 copies/µl. It is used in a total quantity of 5×10^3 copies in every PCR run.

Table 8: A valid PCR run must meet the following conditions:

Sample	Result	ICR Ct	Target gene Ct
Positive control	Positive	N/A *[1]	See Quality Assurance Certificate
Negative control	Negative	Ct > 20	Not detectable

[1] A Ct value for the ICR is not needed to obtain a positive result of the positive control.

The positive and negative controls are valid when they meet the conditions specified in the table. The Ct range for the positive control is specified on the Quality Assurance Certificate included with the product. If one of the two controls does not meet the conditions for a valid run, all the reactions need to be re-analyzed, including the controls.

If the specified values are not met, check the following before repeating the test:

- Expiration date of the reagents used
- Functionality of the devices used
- Correct test procedure

11. Sample interpretation

The results interpretation is done according to table 9.

Table 9: Sample interpretation

Detection of		
SARS-CoV-2	**ICR**	**Result**
Positive	**Positive/ negative**	**SARS-CoV-2 detectable**
Negative	**Positive**	**Target gene not detectable**
Negative	**Negative**	**Invalid**

SARS-CoV-2 is detectable if the sample RNA and the Internal Control RNA show an amplification signal in the detection system.

SARS-CoV-2 is also detectable if the RNA shows an amplification signal, but no amplification signal can be seen for the Internal Control RNA in the detection system. Detecting the Internal Control RNA is not necessary in this case because high amplicon concentrations can result in a weak or absent signal of the Internal Control RNA.

SARS-CoV-2 is not detectable if the RNA shows no amplification signal, but an amplification signal can be seen for the Internal Control RNA in the detection system. Inhibition of the PCR reaction can be ruled out by the detection of the Internal Control RNA.

A sample is invalid if the sample RNA and the Internal Control RNA do not show an amplification signal in the detection system. There are PCR inhibitors in the sample, or an error occurred during the extraction process. The extracted sample should be diluted 1:10 with PCR water and re-amplified, or the isolation and purification of the sample should be improved.

12. Limitations of the method

7. This test is intended only for human respiratory samples.
8. Improper specimen sampling, transport, storage, and handling or a pathogen load below the test's analytical sensitivity can lead to false negative results.
9. The presence of PCR inhibitors can lead to non-evaluable results.
10. Mutations or polymorphisms in the primer or probe binding sites can interfere with the detection of new or unknown variants and can lead to false negative results using RIDA®GENE SARS-CoV-2 RUO.
11. As with all PCR-based *in vitro* tests, extremely low concentrations of the target sequences under the limit of detection (LoD) can be detected. The results obtained are not always reproducible.
12. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the target gene (E gene) is present.

13. Performance characteristics

Not applicable

14. Version history

Version number	Section and designation
2020-02-12	Release version

15. Explanation of symbols

General symbols

RUO	For research use only
	Consult instructions for use
LOT	Lot number
	Expiry
	Store at
REF	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Not applicable

16. References

1. https://www.rki.de/DE/Content/Infekt/Ausbrueche/respiratorisch/Pneumonien-China.html. Accessed on 2020-01-24
2. https://www.spiegel.de/wissenschaft/medizin/covid-19-weltgesundheitsorganisation-verkuendet-neuen-namen-des-coronavirus-a-810ce436-7081-43d2-b8e0-f0b315503e0b Accessed on 2020-02-12
3. https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Risikogebiete.html Accessed on 2020-02-03
4. https://www.rki.de/DE/Content/Infekt/Ausbrueche/respiratorisch/Pneumonien-China.html. Accessed on 2020-02-03

ePlex®
SARS-CoV-2
Assay Manual

RUO

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TABLE OF CONTENTS

INTENDED USE

The ePlex SARS-CoV-2 Test is for research use only (RUO). This test is not for use in diagnostic procedures.

SUMMARY AND EXPLANATION OF TEST

The ePlex SARS-CoV-2 Test is an automated qualitative nucleic acid multiplex *in vitro* diagnostic test for simultaneous detection and identification of SARS-CoV-2 nucleic acid in nasopharyngeal swabs (NPS) collected in viral transport media (VTM). This test is performed on *The True Sample-to-Answer Solution*™ ePlex instrument.

PRINCIPLES OF TECHNOLOGY

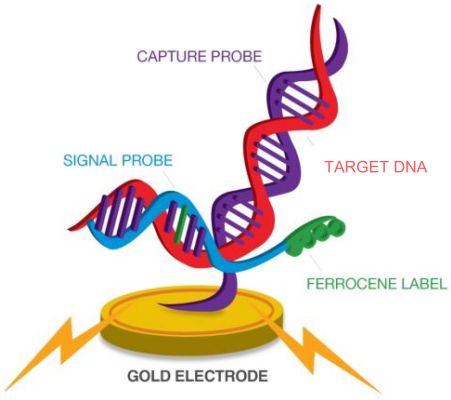
The True Sample-to-Answer Solution ePlex instrument automates all aspects of nucleic acid testing including extraction, amplification, and detection, combining electrowetting and GenMark's eSensor® technology in a single-use cartridge. eSensor technology is based on the principles of competitive DNA hybridization and electrochemical detection, which is highly specific and is not based on fluorescent or optical detection.

Electrowetting, or digital microfluidics, uses electrical fields to directly manipulate discrete droplets on the surface of a hydrophobically coated printed circuit board (PCB). Sample and reagents are moved in a programmable fashion in the ePlex cartridge to complete all portions of the sample processing from nucleic acid extraction to detection.

A sample is loaded onto the ePlex cartridge and nucleic acids are extracted and purified from the specimen via magnetic solid phase extraction. A reverse transcription step is performed to generate complementary DNA from the viral RNA, followed by PCR to amplify the target. Exonuclease digestion creates single-stranded DNA in preparation for eSensor detection.

The target DNA is mixed with ferrocene-labeled signal probes that are complementary to the specific targets on the panel. Target DNA hybridizes to its complementary signal probe and capture probes, which are bound to gold-plated electrodes, as shown below in **Figure 1**. The presence of each target is determined by voltammetry which generates specific electrical signals from the ferrocene-labeled signal probe.

Figure 1: Hybridization complex. Target-specific capture probes are bound to the gold electrodes in the eSensor microarray on the ePlex cartridge. The amplified target DNA hybridizes to the capture probe and to a complementary ferrocene-labeled signal probe. Electrochemical analysis determines the presence or absence of targets using voltammetry.



MATERIALS PROVIDED

Table 1: *The True Sample-to-Answer Solution*™ ePlex SARS-CoV-2 Test Kit Contents

Product	Item number	Components (quantity)	Storage
ePlex SARS-CoV-2 Test	EA008212	ePlex SARS-CoV-2 Test Cartridge (12)	2–8 ºC
		Sample Delivery Device – RP Panel (12)	2–8 ºC

The ePlex SARS-CoV-2 Test reagents are shipped at room temperature; upon receipt, reagents should be stored at 2-8 °C. Safety Data Sheets (SDS) for all reagents provided in this kit may be obtained at https://www.genmarkdx.com/support/safety-data-sheets-sds/. For paper copies, please contact GenMark Customer Service at CustomerService@genmarkdx.com.

REAGENT STORAGE, STABILITY, AND HANDLING

- Store the ePlex SARS-CoV-2 Test kit components at 2–8 ºC.
- Do not open a cartridge pouch until you are ready to perform testing.

MATERIALS NOT PROVIDED

Equipment

- GenMark ePlex instrument and Software
- Pipettes calibrated to deliver 200 µL
- Vortex mixer
- Printer (optional) - See ePlex Operator Manual for compatibility guidelines

Consumables

- Pipette tips, aerosol resistant, RNase/DNase-free
- Disposable, powder free gloves

- 10% bleach for decontamination of appropriate surfaces
- 70% ethanol or isopropyl alcohol

WARNINGS AND PRECAUTIONS

General

- For Research Use Only. Not for use in diagnostic procedures.
- Do not reuse ePlex SARS-CoV-2 Test kit components.
- Do not use a reagent that is damaged.
- Follow the procedure as described in this Assay Manual. Read all instructions before starting the test.

Safety

- Handle all specimens and waste materials as if they were capable of transmitting infectious agents in accordance with Universal Precautions. Observe safety guidelines such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories,* CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*, or other appropriate guidelines.
- Follow routine laboratory safety procedures for handling of reagents (*e.g.*, do not pipette by mouth, wear appropriate protective clothing and eye protection).
- Follow your institution's safety procedures for handling biological samples.
- Dispose materials used in this test, including reagents, specimens, and used vials, in accordance with all federal, state, and local regulations.
- Do not stick fingers or other objects inside the ePlex instrument bays.
- Wash hands thoroughly with soap and water after handling reagents. Launder contaminated clothing prior to re-use.
- Do not puncture or pierce reagent blisters on the ePlex cartridge. Reagents may cause irritation to skin, eyes, and respiratory tract. Harmful if swallowed or inhaled. Contains oxidizing liquids.
- The ePlex SARS-CoV-2 Test cartridge contains chemicals that are classified as hazardous. Review the Safety Data Sheet (SDS) before use, and in cases of exposure, refer to the SDS for more information.

Laboratory

- Specimens should be processed in biosafety cabinets. If a biosafety cabinet is not used, a splash shield or face mask should be used when processing samples.
- A biosafety cabinet that is used for viral or bacterial culture should not be used for sample preparation.
- To mitigate the risk of sample-to-sample contamination, change gloves after dispensing sample into the cartridge.
- Thoroughly decontaminate the lab and all equipment with 10% bleach followed by 70% ethanol or isopropyl alcohol (or equivalent) prior to processing a specimen.
- Contamination of the sample may occur if the sample is loaded in an area where PCR amplicons for respiratory pathogens are generated. Avoid loading sample in areas that are potentially contaminated with PCR amplicon.

PROCEDURE

Procedural Notes

- All frozen samples should be thawed completely before testing.
- Samples should be nasopharyngeal swabs in viral transport media. Please contact GenMark for information on testing other sample types.
- Reagents and cartridge can be used immediately upon removal from 2-8 °C storage. There is no need to equilibrate to room temperature before use.
- Once cartridge is removed from foil pouch, it should be used within 2 hours. Do not open the cartridge pouch until the sample is ready to be tested.
- Once the sample is loaded into the ePlex SARS-CoV-2 Test cartridge, the sample should be tested as soon as possible, or within 2 hours.
- Do not re-use cartridges or Sample Delivery Devices.
- Do not use a Sample Delivery Device that is empty. Visually verify that the vial contains liquid prior to use by tapping vial on the benchtop. Presence of liquid in the vial indicates that the vial can be used for testing. To prevent damage to the Sample Delivery Device, do not centrifuge the Sample Delivery Device.
- Use a new, sterile pipette tip for loading each sample.
- Do not insert a wet cartridge into the ePlex instrument. If the cartridge or sample has leaked, dispose of cartridge in accordance with all federal, state, and local regulations.
- Samples should be transferred into the ePlex SARS-CoV-2 Test cartridge in an amplicon-free, clean environment.
- Samples, consumables, and lab areas should be protected from aerosol or direct contamination with amplicon. Decontaminate laboratory areas and affected equipment with 10% bleach followed by 70% ethanol or isopropyl alcohol (or equivalent).
- To mitigate the risk of sample-to-sample contamination, change gloves after dispensing sample into the cartridge.
- Specimens should be processed in biosafety cabinets. If a biosafety cabinet is not used, a splash shield or face mask should be used when processing samples.
- Dispose materials used in this test, including reagents, specimens, and used vials, in accordance with all regulations.

Detailed Procedure

1. Decontaminate the clean area used for setting up the ePlex SARS-CoV-2 Test with 10% bleach followed by 70% ethanol or isopropyl alcohol (or equivalent).
2. Remove SARS-CoV-2 Test cartridge pouch and Sample Delivery Device from kit packaging.
3. Open the SARS-CoV-2 Test cartridge pouch.
4. Write the accession ID or place a barcode label with accession ID on the SARS-CoV-2 Test cartridge.
5. Write the accession ID or place a barcode label with accession ID on the Sample Delivery Device.
6. Vortex the sample for 3-5 seconds.
7. Gently tap the Sample Delivery Device on the counter or benchtop surface to collect liquid that may have adhered to the sides of the vial.
   NOTE: Contents of vial may adhere to side of vial and inside cap during transit. Visually verify presence of liquid inside vial after tapping vial.
8. Unscrew the purple cap from the Sample Delivery Device.
9. Use a calibrated pipette to aspirate 200 µL of sample and pipette into the Sample Delivery Device.
10. Replace purple cap on Sample Delivery Device. Ensure that cap is securely fastened on the Sample Delivery Device.
11. Vortex the Sample Delivery Device for 10 seconds.
    NOTE: This step should be done immediately before loading sample onto cartridge.

12. Remove the white cover from the tip of the Sample Delivery Device cap.
13. Invert the Sample Delivery Device and dispense the entire volume by squeezing the vial and dispensing the drops into the sample loading port of the SARS-CoV-2 Test cartridge.
    NOTE: Minimize dispensing of bubbles into sample loading port.
14. Close the sample loading port by sliding the cap over the port and firmly pushing down on the cap to securely seal the sample delivery port.
    NOTE: Bubbles can be present when closing the cap.
15. Scan the SARS-CoV-2 Test cartridge using the barcode reader provided with the ePlex instrument.
    NOTE: If an accession ID barcode label is not used, manually enter accession ID with the on-screen keyboard.
    NOTE: The barcode scanner will read both the accession ID barcode (if placed on the cartridge by the operator) and the 2D barcode printed on the cartridge label; however, the barcode scanner will only beep once to indicate that both barcodes have been read.
16. Insert the SARS-CoV-2 Test cartridge into any available bay, indicated by a flashing, white LED light. The test will begin automatically when the cartridge has been inserted into the bay and the pre-run check (cartridge initialization) is completed, indicated by a blue LED light.

QUALITY CONTROL

Internal Controls

Each cartridge includes internal controls that monitor performance of each step of the testing process. A DNA control verifies extraction, amplification, and detection of DNA targets, and RNA controls verify amplification and detection of RNA targets.

In order for a test to be valid, either the internal control or target must generate signal above the defined threshold in the amplification reaction. Internal control results are interpreted by the ePlex software and displayed on ePlex SARS-CoV-2 Test Reports as Internal Control with a result of PASS, FAIL, N/A or INVALID. **Table 5** includes details on the interpretation of Internal Control results.

Table 2: Internal Control Results

Internal Control Result	Explanation	Action
PASS	The internal control or target from the amplification reaction has generated signal above the threshold. The test was completed and internal controls were successful, indicating valid results were generated.	All results are displayed on the SARS-CoV-2 Test Detection Report. Test is valid, report results.
FAIL	Neither the internal control nor target in the amplification reaction generated signal above the threshold. The test was completed but the internal control was not detected, indicating that results are not valid.	No results are displayed on the SARS-CoV-2 Test Report. Test is not valid, repeat the test using a new cartridge.
N/A	The internal control did not generate signal above the threshold, but the target did generate signal above the threshold. The test was completed and the internal control was not successful, however detection of signal above the threshold for the target indicates valid results were generated.	All results are displayed on the SARS-CoV-2 Test Detection Report. Test is valid, report results.

Internal Control Result	Explanation	Action
INVALID	An error has occurred during processing that prevents analysis of signal data. The test has not successfully completed and results for this test are not valid. This is often due to an instrument or software error.	No results are displayed on the SARS-CoV-2 Test Detection Report. Test is not valid, repeat the test using a new cartridge.

External Controls

Positive and negative external controls should be tested as part of good laboratory practice, in accordance with the appropriate accrediting organization as applicable and following the user's laboratory standard quality control procedures. Viral transport medium can be used as the negative control. Previously characterized positive samples or viral transport medium spiked with well characterized organism can be used as the external positive control. External controls should be run in accordance with laboratory protocols and accrediting organizations, as applicable.

RESULTS

Table 3: Interpretation of Results on the ePlex SARS-CoV-2 Test Detection Report

Target Result	Explanation	Action
Target Detected	The test was completed successfully, and the target has generated signal above its defined threshold, and the Internal Control was reported as PASS.	All results are displayed on the SARS-CoV-2 Test Detection Report. Test is valid, report results.
Target Not Detected	The test was completed successfully, and the target did not generate signal above its defined threshold, and the Internal Control was reported as PASS.	All results are displayed on the SARS-CoV-2 Test Detection Report. Test is valid, report results.
Invalid	The test has not successfully completed, and results for this test are not valid. This is often due to an instrument or software error or failure of an internal control.	No results are displayed on the SARS-CoV-2 Test Detection Report. Test is not valid, repeat test.

TEST REPORTS

There are several different reports that are available on the ePlex instrument. Results are provided in a printable format, may be viewed electronically, or may be exported for additional analysis. Reports can be customized with account specific information such as the address, logo, and institution specific footers on each report. For more information on ePlex reports, refer to the ePlex Operator Manual.

Detection Report

The SARS-CoV-2 Test Detection Report includes the results for each individual sample run on the ePlex instrument.

The Summary section indicates the overall test result. The Results section includes a list of all targets on the panel with an individual result for each. Results for each target are reported as Detected, Not Detected, or Invalid (displayed as a red **x**); results for the Internal Control are reported as PASS, FAIL, INVALID, or N/A.

External Control Report

The SARS-CoV-2 Test External Control Report is generated for an external control that has been pre-defined in the ePlex RP Panel software. For more information on defining external controls on the ePlex SARS-CoV-2 Test, refer to the ePlex Operator Manual.

The Summary section indicates the overall result (Pass or Fail status) for that external control. The Results section includes a list of all panel targets with the result, expected result, and Pass/Fail status for each. Results are reported as Detected, Not Detected, or Invalid (displayed as a red **x**). A target is reported as Pass if the actual result matches the expected result (as defined for that control); a target is reported as Fail if the actual result does not match the expected result. If the actual results for each target match the expected result for each target (all targets reported as Pass), the overall result for the external control is reported as Pass in the Summary section. If the actual result for any target does not match the expected result, the overall result for the external control is reported as Fail in the Summary section.

Summary Report

The Summary Report allows the operator to use defined searchable criteria to create customized reports, using specified targets, dates, range of dates, sample, external control, test bay, or operator. For more information on creating Summary Reports, refer to the ePlex Operator Manual.

TROUBLESHOOTING

Table 4: Troubleshooting Table

For a complete list of all ePlex error messages and a description of the messages, please refer to the ePlex Operator Manual.

Error	Error Messages	Description	Re-test Recommendations
Test did not start	Cartridge failure The cartridge initialization test failed Cartridge not present Bay heater failure Unknown error Bay main / fluid motor failure EEPROM failure Bay over pressured Bay temperature out of range The system was unable to read the cartridge Cartridge inserted doesn't match the serial number of the cartridge scanned	An error that occurs during pre-run checks (cartridge initialization) of the cartridge upon insertion into the bay. Cartridge initialization occurs when the cartridge is first inserted into the bay and takes approximately 90 seconds. Upon completion of cartridge initialization, the cartridge cannot be restarted, but prior to this point, the cartridge can be restarted. To verify cartridge initialization has completed, examine the cartridge label upon removal from the bay. If the cartridge label has been pierced, the test has already started and	1. Remove cartridge from bay. a. Reset bay to clear the error b. Restart cartridge in any available bay 2. If the cartridge is not able to be run on the second try and again generates an error during cartridge initialization, this indicates an issue with the cartridge. This cartridge should be discarded following laboratory procedures and the sample should be repeated using a new cartridge. Bay(s) should be reset to clear the errors. Please contact MAS or GenMark Technical Support to alert them of the issue. If the bay remains in an error state (flashing red) after the cartridge has been removed, then the bay must be

Error	Error Messages	Description	Re-test Recommendations
	The system is not ready to accept the cartridge The system was unable to enable cartridge insertion for the bay The system failed to prepare the cartridge for processing	cartridge cannot be reused. If the label has not been pierced, follow the recommendation as stated.	reset through the Bay Configuration menu before it can be used to run cartridges.
Test did not finish	Bay heater failure Bay main / fluid motor failure Bay voltage failure Bay sub-system communication timeout Cartridge failure Bay over pressured Bay auto-calibration failure Bay temperature out of range The system was unable to eject the cartridge from the bay	This type of error occurs during the run, after pre-run checks (cartridge initialization) have finished, and prevents the cartridge from being processed to completion.	Reagents have been consumed and the cartridge cannot be reused. Contact GenMark Technical Support and proceed with repeat testing of the sample using a new cartridge. If the bay remains in an error state (flashing red) after the cartridge has been removed, then the bay must be reset through the Bay Configuration menu before it can be used to run cartridges.
Invalid		This is an error that results in no valid results being generated. A test report will be generated, but all targets and the internal control will be invalid.	Reagents have been consumed and the cartridge cannot be reused. Contact GenMark Technical Support and proceed with repeat testing of the sample using a new cartridge.

Technical Support

GenMark Technical support is available 24 hours a day, 7 days a week to provide the highest level of customer support and satisfaction.

GenMark Diagnostics, Inc.
5964 La Place Court
Carlsbad, CA 92008 USA
Phone: 1 800 eSensor (1 800 373 6767), Option 2
Email: technicalsupport@genmarkdx.com

GLOSSARY OF SYMBOLS

Symbol	Description	Symbol	Description
	Batch Code		Date of Manufacture YYYY-MM-DD
	Caution		Serial number
	Contains sufficient for <n> tests		Catalog number

Symbol	Description	Symbol	Description
	Consult instructions for use		Biological risks
	Temperature range		Upper limit of temperature
	Lower limit of temperature		Cartridge Lot
	Manufacturer		Irritant, dermal sensitizer, acute toxicity (harmful), narcotic effects, respiratory tract irritation
	Oxidizers		Carcinogen, Respiratory Sensitizer, Reproductive Toxicity, Target Organ Toxicity, Mutagenicity, Aspiration Toxicity

TRADEMARKS

GenMark®, GenMark Dx®, eSensor®, ePlex®, are registered trademarks of GenMark Diagnostics, Inc. The True Sample-to-Answer Solution™ is a trademark of GenMark Diagnostics, Inc.

PATENT INFORMATION

ePlex® SARS-CoV-2 Test and/or use thereof features technology claimed in one or more of the following United States and European patents owned or licensed by GenMark Diagnostics Inc. or its subsidiaries, with multiple additional foreign and domestic patents pending: U.S. Patent Nos. 6,753,143, 7,172,897, 7,312,087, 7,534,331, 7,820,391, 8,486,247, 9,222,623, 9,410,663, 9,453,613, 9,498,778, 9,500,663, 9,598,722; 9,874,542, 9,957,553, 10,001,476, International Patent Nos. 1218541, 1246699, 60125713.8, 2220102, 602008031596.7, 1246699, 2278757, 60125713.8, 3548159, 9874542, 60017809.9, 1350568, 3548159, 2965817; and other international counterparts.

Unless otherwise agreed to in writing, by using a cartridge, Recipient acknowledges that Recipient has read, accepts and agrees to be bound by and comply with the General Terms and Conditions of Sale available on GenMark's website which can be amended from time to time by GenMark without consent. If Recipient does not accept and agree to be bound by the General Terms and Conditions of Sale, Recipient will immediately cease any further use of the cartridge.

This product is subject to a limited license to use the product in the field of human *in vitro* diagnostics and research reasonably related thereto. Users are prohibited from using this product for other applications, including in the field of forensics (including human identification testing).

Effective Date: February 2020

Clinical Micro Sensors, Inc. dba GenMark Diagnostics, Inc.
5964 La Place Court, Carlsbad, CA 92008
+1 760 448 4300
www.genmarkdx.com

REF 130219016M: 100 tests

MAGLUMI™ 2019-nCoV IgM (CLIA)

INTENDED USE
The kit is an *In Vitro* chemiluminescence immunoassay for the qualitative determination of IgM antibodies to novel coronavirus (2019-nCoV IgM) in human serum or plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

SUMMARY AND EXPLANATION OF THE TEST
The novel coronavirus (2019-nCoV) causes an epidemic of acute respiratory syndrome in humans in Wuhan[1], belonging to the genus Betacoronavirus. It has an envelope, particles are round or oval, often polymorphic, and the diameter is 60 ~ 140nm. Its genetic characteristics are significantly different from SARSr-CoV and MERSr-CoV. Current research shows that it has more than 85% homology with bat SARS-like coronavirus (bat-SL-CoVZC45)[2].
2019-nCoV is mainly transmitted through respiratory droplets and can also be transmitted through contact. The sources of infection seen so far are mainly patients with pneumonia infected by the novel coronavirus[2].
Research has shown that detection of IgM and IgG antiviral antibodies in the serum samples from a patient[3]. After human infection in 2019-nCoV, its antigen stimulates the immune system to produce an immune response, and corresponding antibodies appear in the blood. Among them, 2019-nCoV IgM appears earlier, and then 2019-nCoV IgM titers decrease, the 2019-nCoV IgG potency rose rapidly.
This kit is mainly used for the assisted diagnosis of the novel coronavirus (2019-nCoV) infection.
2019-nCoV, named by the World Health Organization on January 7, 2020, is announced the official name: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) on February 11, 2020. On the same day, the Director-General of the World Health Organization (WHO) Tan Desai announced that pneumonia infected with SARS-CoV-2 will be officially named "COVID-19".

PRINCIPLE OF THE TEST
The MAGLUMI 2019-nCoV IgM (CLIA) assay is a capture chemiluminescence immunoassay.
The prediluted sample (or calibrator/control, if applicable), buffer, magnetic microbeads coated with anti-human IgM monoclonal antibody are mixed thoroughly and incubated, forming immune-complexes. After precipitation in a magnetic field, decant the supernatant, and perform a wash cycle. Then add 2019-nCoV recombinant antigen labeled with ABEI and incubate to form complexes. After precipitation in a magnetic field, decant the supernatant, and then perform another wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of 2019-nCoV IgM present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS
Material Provided

Component	Contents	100 tests (REF: 130219016M)
Magnetic Microbeads	Magnetic microbeads coated with anti-human IgM monoclonal antibody, PBS buffer and BSA, NaN_3 (<0.1%).	2.5 mL
Calibrator Low	2019-nCoV IgM, PBS buffer and BSA, NaN_3 (<0.1%).	1.0 mL
Calibrator High	2019-nCoV IgM, PBS buffer, and BSA, NaN_3 (<0.1%).	1.0 mL
Buffer	PBS buffer, Goat anti-Human IgG, Goat anti-Human IgA Mouse IgG, Goat IgG and BSA, NaN_3 (<0.1%).	23.5 mL
ABEI Label	2019-nCoV recombinant antigen labeled with ABEI, Tris-HCl buffer, Mouse IgG, Goat IgG, and BSA, NaN_3 (<0.1%).	23.5 mL
Diluent	PBS buffer, Goat anti-Human IgG, Goat anti-Human IgA Mouse IgG, Goat IgG and BSA, NaN_3 (<0.1%).	23.5 mL
Negative Control	PBS buffer, containing BSA, NaN_3 (<0.1%).	1.0 mL
Positive Control	2019-nCoV IgM, PBS buffer, containing BSA and NaN_3 (<0.1%).	1.0 mL
All reagents are provided ready-to-use.		

Accessories Required But Not Provided
MAGLUMI Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M;130299012M; 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101
Maglumi 600	REF: 23020018
Maglumi 800	REF: 23020003
Maglumi 1000	REF: 23020009
Maglumi 2000	REF: 23020006
Maglumi 2000 Plus	REF: 23020007
Maglumi 4000	REF: 23020014
Maglumi 4000 Plus	REF: 23020037
MAGLUMI X8	REF: 010101008801
Biolumi 8000	REF: 23010001

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.

CALIBRATION
Traceability: This method has been standardized against the SNIBE internal reference substance.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:
- After each exchange of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used.
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL
Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI system. For instructions for use and target value refer to ***2019-nCoV IgM Quality Control Information***. User needs to judge results with their own standards and knowledge.

For details about entering quality control values, refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.
To monitor system performance, quality control materials (negative control and positive control) are required. Treat all quality control samples with the same level of care as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, measurement of the quality control should be repeated. If the quality control results still do not fall within the range, do not report results and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instruction for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Human serum or plasma may be used with the 2019-nCoV IgM (CLIA) assay. Serum including samples collected using standard sampling tubes, tubes containing separating gel or procoagulant inert separation tubes. For plasma samples, the anticoagulants including K_2-EDTA, K_3-EDTA, Na_2 -EDTA, have been tested and may be used with this assay.
- Do not use grossly hemolyzed specimens.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Specimens removed from the separator gel, cells or clot may be stored 5 days at 2-8°C.
- Specimens can be stored more than 5 days frozen at -70°C or colder. Avoid repeated freezing and thawing. Frozen specimens must be mixed thoroughly after thawing by low speed vortexing or by gently inverting.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at ≥ 10,000RCF (Relative Centrifugal Force) for 10 minutes. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Before shipping specimens, it is recommended that specimens be removed from the separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen..
- The sample volume required for a single determination is 10 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and container must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets, which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the Reagent Kit on the system for the first time, the Reagent Kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are "single use", and if more seals are needed, please contact Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Store at 2-8°C. Do not freeze.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

Stability of the reagent	
unopened at 2-8°C	until the stated expiration date
opened at 2-8°C	6 weeks
onboard	4 weeks

TEST PROCEDURE

Preparation of the Reagent

- Take the reagent kit out of the box and observe the sealing film and other parts of the reagent kit to see if there is any leakage. In case of leakage, please contact your local agent immediately. And then tear off the kit sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Click **<Calibration>** or **<Batch Calibration>** button to execute calibration operation; For specific information on ordering calibrations, refer to the Calibration Section of the Operating Instructions.
- Execute recalibration according to the calibration interval required in this manual.

Quality Control

- In order to avoid manually error in entry of QC information, the provided barcode labels of quality control in the kit can be used attached on the test tubes.
- Strictly follow the quality control procedures when using the quality controls..
- If users do not use the provided barcode labels for positive and negative controls contained within the packaging, then quality controls should be ordered manually.
- For specific information on ordering quality controls, refer to the Quality Control Section of the Operating Instructions.

Sample Testing

- Order the samples in the Sample Area of the software and click the **<Start>** button to execute testing. For specific information on ordering patient specimens, refer to the Sample Ordering Section of the Operating Instructions.

To ensure proper test performance, strictly adhere to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

DILUTION

Samples with concentrations above 30.0 AU/mL can be diluted automatically by analyzers or manually. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzer, ,the analyzer software automatically takes the dilution into account when calculating the sample concentration.

The automatic sample dilution is available after dilution settings are done in the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

LIMITATIONS

- This test is suitable only for investigating single samples, not for pooled samples or heat-inactivated specimens.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test results.
- Fresh sample is recommended. If a low positive result was get, repeated test should be conducted after centrifuged especial severely or using additional test to confirm the result.
- Assay results should be utilized in conjunction with other clinical and laboratory methods to assist the clinician in making individual patient diagnostic decisions.
- If the 2019-nCoV IgM results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- HAMA antibodies in test samples may cause interference in immunoassays.

RESULTS

Calculation of Results

The analyzer automatically calculates the concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in AU/mL. For further information please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Interpretation of Results

Results obtained with the 2019-nCoV IgM assay can be interpreted as follows:

- Non-reactive: A result less than 0.900 AU/mL (<0.900 AU/mL) is considered to be non-reactive.
- Gray zone: A result in the interval between 0.900 and 1.10 (0.900≤ x<1.10 AU/mL) is considered to be equivocal.
- Reactive: A result greater than or equal to 1.10 AU/mL (≥1.10 AU/mL) is considered to be reactive.

PERFORMANCE CHARACTERISTICS

Precision

Precision for 2019-nCoV IgM assay was determined as described in the CLSI EP5-A3. 2 controls and 3 samples containing different concentration of analyte were assayed in duplicate at three sites on five days, with 3 runs per day, one lot of reagent for each run and 2 replicates per run. The result is summarized in the following table:

Sample	Mean Value (AU/mL)	N	Repeatability		Between-Lot		Between-Day		Between-Site		Reproducibility	
			SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
NQC	0.296	90	0.025	NA	0.011	NA	0.003	NA	0.019	NA	0.034	NA
PQC	3.911	90	0.164	4.19	0.062	1.59	0.062	1.59	0.293	7.49	0.347	8.87
S1	0.501	90	0.048	NA	0.009	NA	0.007	NA	0.023	NA	0.055	NA
S2	3.517	90	0.162	4.61	0.053	1.51	0.041	1.17	0.054	1.54	0.183	5.20
S3	14.710	90	0.269	1.83	0.072	0.49	0.127	0.86	0.589	4.00	0.664	4.51

Endogenous interference

Two serum samples (one negative sample, one positive sample) were spiked with potential endogenous interference. The results of the interferences are listed in the following table:

Interference	No interference up to
Hemoglobin	2000 mg/dL
Bilirubin	40 mg/dL
Triglycerides	1000 mg/dL
Rheumatoid Factor	1500 IU/mL
HAMA	30 ng/mL

Drug interference

Two serum samples (one negative sample, one positive sample) were spiked with potential endogenous interference. The results of the interferences are listed in the following table:

Interference	No interference up to
Acetylcysteine	15 mg/dL
Ampicillin sodium	100 mg/dL
Cefoxitin	250 mg/dL
Metronidazole	20 mg/dL
Tetracycline	5 mg/dL
Aspirin	100 mg/dL
Rifampin	6 mg/dL
Acetaminopthen	20 mg/dL
Ibuprofen	50 mg/dL
Theophylline	10 mg/dL
Lamivudine	30 mg/dL
Entecavir	0.5 mg/L
Telbivudine	60 mg/dL
Adefovir	1 mg/dL

Analytical specificity

Clinical 2019-nCoV IgM negative samples, which contain potential cross-reactants including influenza virus type A antibody, influenza virus type B antibody, parainluenza virus antibody, respiratory syncytial virus antibody, adenovirus antibody, EBV NA IgG, EBV VCA IgM/IgG, CMV IgM/IgG, M.Pneumonia IgM/IgG, chlamydia pneumoniae IgM/IgG, Candida albicans, ANA were used to evaluate the cross-reactivity of 2019-nCoV IgM assay. Of all the potential cross-reactants, none were found to cause false positive in the 2019-nCoV IgM assay.

Clinical Sensitivity

The clinical sensitivity was determined for 87 confirmed novel coronavirus infected specimens. The clinical sensitivity was calculated to be 48.28%.

Specimen Category	2019-nCoV IgM (CLIA)		
	N	Positive	%Sensitivity
Clinical confirmed positive samples	87	42	48.28%

Clinical specificity

The clinical specificity was determined for 370 non- novel coronavirus infected specimens, normal samples and interference samples. The clinical specificity was calculated to be 100%.

Specimen Category	2019-nCoV IgM (CLIA)		
	N	Negative	%Specificity
negative specimens	370	370	100%

REFERENCES

1. Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature (2020). https://doi.org/10.1038/s41586-020-2012-7.
2. Diagnosis and treatment of pneumonitis caused by novel coronavirus (version 4).
3. Na Zhu, Ph.D., Dingyu Zhang, et al.A Novel Coronavirus from Patients with Pneumonia in China, 2019[J].New England Journal of Medicine, 2020.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
8 °C 2 °C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for <n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code

REF 130219015M: 100 tests

MAGLUMI™ 2019-nCoV IgG (CLIA)

INTENDED USE
The kit is an *In Vitro* chemiluminescence immunoassay for the qualitative determination of IgG antibodies to novel coronavirus (2019-nCov IgG) in human serum or plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

SUMMARY AND EXPLANATION OF THE TEST
The novel coronavirus (2019-nCoV) causes an epidemic of acute respiratory syndrome in humans in Wuhan[1], belonging to the genus Betacoronavirus. It has an envelope, particles are round or oval, often polymorphic, and the diameter is 60 ~ 140nm. Its genetic characteristics are significantly different from SARSr-CoV and MERSr-CoV. Current research shows that it has more than 85% homology with bat SARS-like coronavirus (bat-SL-CoVZC45)[2].
2019-nCoV is mainly transmitted through respiratory droplets and can also be transmitted through contact. The sources of infection seen so far are mainly patients with pneumonia infected by the novel coronavirus.[2]
Research has shown that detection of IgM and IgG antiviral antibodies in the serum samples from a patient[3]. After human infection in 2019-nCoV, its antigen stimulates the immune system to produce an immune response, and corresponding antibodies appear in the blood. Among them, 2019-nCoV IgM appears earlier, and then 2019-nCoV IgM titers decrease, the 2019-nCoV IgG potency rose rapidly.
This kit is mainly used for the assisted diagnosis of the novel coronavirus (2019-nCoV) infection.
2019-nCoV, named by the World Health Organization on January 7, 2020, is announced the official name: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV) on February 11, 2020. On the same day, the Director-General of the World Health Organization (WHO) Tan Desai announced that pneumonia infected with SARS-CoV-2 will be officially named "COVID-19".

PRINCIPLE OF THE TEST
The MAGLUMI 2019-nCoV IgG (CLIA) assay is an indirect chemiluminescence immunoassay.
The prediluted sample (or calibrator/control, if applicable), buffer and magnetic microbeads coated with 2019-nCoV recombinant antigen are mixed thoroughly and incubated, forming immune-complexes. After precipitation in a magnetic field, decant the supernatant, and perform a wash cycle. Then add ABEI labeled with anti-human IgG antibody, and incubate to form complexes. After precipitation in a magnetic field, decant the supernatant, and perform another wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of 2019-nCoV IgG presented in the sample (or calibrator/control, if applicable).

KIT COMPONENTS
Material Provided

Component	Contents	100 tests (REF: 130219015M)
Magnetic Microbeads	Magnetic microbeads coated with 2019-nCoV recombinant antigen, PBS buffer and BSA, NaN_3(<0.1%).	2.5 mL
Calibrator Low	2019-nCoV IgG , PBS buffer and BSA, NaN_3(<0.1%).	1.0 mL
Calibrator High	2019-nCoV IgG, PBS buffer and BSA, NaN_3(<0.1%).	1.0 mL
Buffer	NaCl and BSA, NaN_3(<0.1%).	23.5 mL
ABEI Label	Anti-human IgG antibody labeled with ABEI, Tris-HCl buffer, Mouse IgG, Goat IgG, and BSA, NaN_3 (<0.1%).	23.5 mL
Diluent	PBS buffer and BSA, NaN_3(<0.1%).	23.5 mL
Negative Control	PBS buffer, containing BSA, NaN_3 (<0.1%).	1.0 mL
Positive Control	2019-nCoV IgG, PBS buffer,containing BSA and NaN_3 (<0.1%).	1.0 mL
All reagents are provided ready-to-use.		

Accessories Required But Not Provided
MAGLUMI Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M;130299012M; 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101
Maglumi 600	REF: 23020018
Maglumi 800	REF: 23020003
Maglumi 1000	REF: 23020009
Maglumi 2000	REF: 23020006
Maglumi 2000 Plus	REF: 23020007
Maglumi 4000	REF: 23020014
Maglumi 4000 Plus	REF: 23020037
MAGLUMI X8	REF: 010101008801
Biolumi 8000	REF: 23010001

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.

CALIBRATION
Traceability: This method has been standardized against the SNIBE internal reference substance.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:
- After each exchange of lots (Reagent or Starter 1+2).
- Every week and/or each time a new reagent kit is used.
- After instrument service is required.
- If controls lie outside the expected range.

QUALITY CONTROL
Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI system. For instructions for use and target value refer to ***2019-nCoV IgG Quality Control Information***. User needs to judge results with their own standards and knowledge.
For details about entering quality control values, refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence

immunoassay analyzer.
To monitor system performance, quality control materials (negative control and positive control) are required. Treat all quality control samples with the same level of care as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, measurement of the quality control should be repeated. If the quality control results still do not fall within the range, do not report results and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instruction for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical support provider or distributor for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Human serum or plasma may be used with the 2019-nCoV IgG (CLIA) assay. Serum including samples collected using standard sampling tubes, tubes containing separating gel or procoagulant inert separation tubes. For plasma samples, the anticoagulants including K_2-EDTA, K_3-EDTA, Na_2 -EDTA, have been tested and may be used with this assay.
- Do not use grossly hemolyzed specimens.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Specimens removed from the separator gel, cells or clot may be stored 5 days at 2-8°C.
- Specimens can be stored more than 5 days frozen at -70°C or colder. Avoid repeated freezing and thawing. Frozen specimens must be mixed thoroughly after thawing by low speed vortexing or by gently inverting.
- For optimal results, specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give inconsistent results and must be transferred to a centrifuge tube and centrifuged at ≥ 10,000RCF (Relative Centrifugal Force) for 10 minutes. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.
- Before shipping specimens, it is recommended that specimens be removed from the separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination is 10 µL.

WARNING AND PRECAUTIONS FOR USERS

- **IVD** For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION:** This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and container must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets, which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the Reagent Kit on the system for the first time, the Reagent Kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are "single use", and if more seals are needed, please contact Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Store at 2-8°C. Do not freeze.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

Stability of the reagent	
unopened at 2-8°C	until the stated expiration date
opened at 2-8°C	6 weeks
onboard	4 weeks

TEST PROCEDURE

Preparation of the Reagent

- Take the reagent kit out of the box and observe the sealing film and other parts of the reagent kit to see if there is any leakage. In case of leakage, please contact your local agent immediately. And then tear off the kit sealing film carefully.
- Open the reagent area door; Hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Click **<Calibration>** or **<Batch Calibration>** button to execute calibration operation; For specific information on ordering calibrations, refer to the Calibration Section of the Operating Instructions.
- Execute recalibration according to the calibration interval required in this manual.

Quality Control

- In order to avoid manually error in entry of QC information, the provided barcode labels of quality control in the kit can be used attached on the test tubes.
- Strictly follow the quality control procedures when using the quality controls.
- If users do not use the provided barcode labels for positive and negative controls contained within the packaging, then quality controls should be ordered manually.
- For specific information on ordering quality controls, refer to the Quality Control Section of the Operating Instructions.

Sample Testing

- Order the samples in the Sample Area of the software and click the **<Start>** button to execute testing. For specific information on ordering patient specimens, refer to the Sample Ordering Section of the Operating Instructions.

To ensure proper test performance, strictly adhere to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay

analyzer.

DILUTION

The automatic sample dilution is available after dilution settings are done in the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer. The recommended dilution factor is 20 times (1:19, 1part sample with19 parts diluent).After automatic dilution, multiply the result by the dilution factor.

LIMITATIONS

- This test is suitable only for investigating single samples, not for pooled samples or heat-inactivated specimens.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test results.
- Fresh sample is recommended. If a low positive result was get, repeated test should be conducted after centrifuged especial severely or using additional test to confirm the result.
- Assay results should be utilized in conjunction with other clinical and laboratory methods to assist the clinician in making individual patient diagnostic decisions.
- If the 2019-nCoV IgG results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- HAMA antibodies in test samples may cause interference in immunoassays.

RESULTS

Calculation of Results

The analyzer automatically calculates the concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in AU/mL. For further information please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Interpretation of Results

Results obtained with the 2019-nCoV IgG assay can be interpreted as follows:

- Non-reactive: A result less than 0.900 AU/mL (<0.900 AU/mL) is considered to be non-reactive.
- Gray zone: A result in the interval between 0.900 and 1.100(0.900≤ x<1.10 AU/mL) is considered to be equivocal.
- Reactive: A result greater than or equal to 1.10 AU/mL (≥1.10 AU/mL) is considered to be reactive.

PERFORMANCE CHARACTERISTICS

Precision

Precision for 2019-nCoV IgG assay was determined as described in the CLSI EP5-A3.2 controls and 3 human serum pools containing different concentration of analyte were assayed in duplicate at three sites on five days, with 3 runs per day, one lot of reagent for each run and 2 replicates per run. The result is summarized in the following table:

Sample	Mean Value (AU/mL)	N	Repeatability		Between-Lot		Between-Day		Between-Site		Reproducibility	
			SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV	SD (AU/mL)	%CV
NQC	0.293	90	0.024	NA	0.005	NA	0.008	NA	0.023	NA	0.035	NA
PQC	3.915	90	0.199	5.08	0.069	1.76	0.032	0.82	0.265	6.77	0.340	8.68
S1	0.491	90	0.043	NA	0.015	NA	0.004	NA	0.013	NA	0.047	NA
S2	3.486	90	0.212	6.08	0.060	1.72	0.050	1.43	0.071	2.04	0.237	6.80
S3	9.807	90	0.159	1.62	0.122	1.24	0.082	0.84	0.639	6.52	0.675	6.88

Endogenous interference

Two serum samples (one negative sample, one positive) were spiked with potential endogenous interference. The results of the interferences are listed in the following table:

Interference	No interference up to
Hemoglobin	2000 mg/dL
Bilirubin	40 mg/dL
Triglycerides	1000 mg/dL
Rheumatoid Factor	1500 IU/mL
HAMA	30 ng/mL

Drug interference

Two serum samples (one negative sample, one positive) were spiked with potential endogenous interference. The results of the interferences are listed in the following table:

Interference	No interference up to
Acetylcysteine	15 mg/dL
Ampicillin sodium	100 mg/dL
Cefoxitin	250 mg/dL
Metronidazole	20 mg/dL
Tetracycline	5 mg/dL
Aspirin	100 mg/dL
Rifampin	6 mg/dL
Acetaminopthen	20 mg/dL
Ibuprofen	50 mg/dL
Theophylline	10 mg/dL
Lamivudine	30 mg/dL
Entecavir	0.5 mg/L
Telbivudine	60 mg/dL
Adefovir	1 mg/dL

Analytical specificity

Clinical 2019-nCoV IgG negative samples, which contain potential cross-reactants including Influenza virus type A antibody, Influenza virus type B antibody, parainluenza virus antibody, respiratory syncytial virus antibody, adenovirus antibody, EBV NA IgG, EBV VCA IgM/IgG, CMV IgM/IgG, M.Pneumonia IgM/IgG, chlamydia pneumoniae IgM/IgG, Candida albicans, ANA were used to evaluate the cross-reactivity of 2019-nCoV IgG assay. Of all the potential cross-reactants, none were found to cause false positive in the 2019-nCoV IgG assay.

Clinical Sensitivity

The clinical sensitivity was determined for 91 confirmed novel coronavirus infected specimens. The clinical sensitivity was calculated to be 91.21%.

Specimen Category	2019-nCoV IgG (CLIA)		
	N	Positive	%Sensitivity
Clinical confirmed positive samples	91	83	91.21%

Clinical specificity

The clinical specificity was determined for 370 non- novel coronavirus infected specimens, normal samples and interference samples. The clinical specificity was calculated to be 100%.

Specimen Category	2019-nCoV IgG (CLIA)		
	N	Negative	%Specificity
negative specimens	370	370	100%

REFERENCES

1.Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature (2020). https://doi.org/10.1038/s41586-020-2012-7.

2. Diagnosis and treatment of pneumonitis caused by novel coronavirus (version 4).

3. Na Zhu, Ph.D., Dingyu Zhang, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019[J]. New England Journal of Medicine, 2020.

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EC REP

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
8 °C 2 °C	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for <n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code