RIDA®GENE SARS-CoV-2 RUO

REF PG6815RUO

R-Biopharm AG, An der neuen Bergstrasse 17, 64297 Darmstadt, Germany
Phone: +49 (0) 61 51 81 02-0, Fax: +49 (0) 61 51 81 02-20

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

1. This test is intended only for human respiratory samples.
2. Improper specimen sampling, transport, storage, and handling or a pathogen load below the test's analytical sensitivity can lead to false negative results.
3. The presence of PCR inhibitors can lead to non-evaluable results.
4. 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.
5. 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.
6. 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