RIDA®GENE Flu & SARS-CoV-2

REF PG6825

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1. Intended use

For in vitro diagnostic use. The RIDA®GENE Flu & SARS-CoV-2 test, performed on the LightCycler® 480II real-time PCR instrument, is a multiplex real-time RT-PCR for the direct qualitative detection and differentiation of flu A/flu B and coronavirus (SARS-CoV-2) RNA in human nasal/throat swabs from persons with signs and symptoms of respiratory infection.

The RIDA®GENE Flu & SARS-CoV-2 test is intended to support the differential diagnosis of flu A/flu B and SARS-CoV-2 infections in patients with symptoms of respiratory infection in connection with other clinical and laboratory findings.

Negative results do not rule out infection with flu A/flu B or SARS-CoV-2 and should not be used as the sole basis for diagnosis. The product is intended for use by professionals working in hospital laboratories, reference laboratories, private laboratories, or public laboratories.

2. Summary and explanation of the test

At the end of December 2019 in the Chinese metropolis of Wuhan, numerous cases of pneumonia of unknown cause occurred.[1] At the beginning of January 2020, Chinese authorities identified a novel coronavirus (SARS-CoV-2) as the cause of these illnesses.[1] The disease caused by SARS-CoV-2 was officially named COVID-19 (“coronavirus disease 2019”) and is transmitted from human to human.[2] Due to this pathogen’s novelty, the epidemic rapidly evolved into a pandemic.

There have already been 105,805,951 cases recorded worldwide (as of February 09, 2021).[3] At the end of January 2020, the first cases were confirmed in Germany as well. Germany has had 2,291,924 cases so far (as of February 09, 2021).[4] The WHO declared a Public Health Emergency of International Concern on 2020-01-31.[1]

SARS-CoV-2 and influenza viruses share some similarities. For instance, the primary route of transmission for both of these pathogens is the airborne route through virus-laden respiratory droplets generated by breathing, coughing, talking, and sneezing.[5,6] The symptoms in the early stage are typical for respiratory viral pathogens. The most commonly reported symptoms for both pathogens are fever, cough, and nasal congestion. Since SARS-CoV-2 disease courses can vary greatly in symptoms and severity (from asymptomatic to severe pneumonia with lung failure and death), the differentiation of SARS-CoV-2 and influenza viruses is important for further therapy.

3. Test principle

RIDA®GENE Flu & SARS-CoV-2 is a multiplex real-time RT-PCR test for the direct qualitative detection and differentiation of flu A/flu B and coronavirus (SARS-CoV-2) RNA in human nasal/throat swabs.
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. Next, real-time PCR is used to amplify the gene fragments for influenza A (M gene), influenza B (NP1 gene), and SARS-CoV-2 (E gene and RdRp gene). The amplified target sequences are detected using hydrolysis probes that are labeled with a quencher at one end and a fluorescent reporter dye (fluorophore) at the other. The probes hybridize to the amplicon in the presence of a target sequence. During extension, 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 Flu & SARS-CoV-2 test contains an Internal Control RNA (ICR) to be able to control sample preparation and/or potential PCR inhibition.

4. Reagents provided

Table 1: Reagents provided (The reagents provided in the kit are sufficient for 200 determinations.)

Kit code	Reagent	Amount		Lid color
1	Reaction Mix	4x	1050 µl	Yellow
2	Enzyme Mix	1x	160 µl	Red
R	Internal Control RNA	4x	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 to 8 °C).
- Repeated freezing/thawing of up to 20 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 to 8 °C).

6. Reagents required but not provided

The RIDA®GENE Flu & SARS-CoV-2 multiplex real-time RT-PCR test was verified using the following combination of extraction platform and real-time PCR device:

Tab.2a: Necessary equipment (verified)

Extraction platform	
Promega	Maxwell® RSC
Real-time PCR device	
Roche	LightCycler® 480II

Also, the RIDA®GENE Flu & SARS-CoV-2 multiplex real-time RT-PCR test is compatible for use with the following real-time PCR devices:

Table 2b: Necessary equipment (compatible)

Real-time PCR devices	
R-Biopharm	RIDA®CYCLER
Applied Biosystems	ABI 7500 Fast Dx
Bio-Rad	CFX96™ Dx
QIAGEN	Rotor-Gene Q
qTOWER³	Analytik Jena
PCR Setup	
CyBio FeliX	Analytik Jena

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

Should you have to use other extraction procedures or real-time PCR devices, 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, reaction vials, films)
- Centrifuge with rotor for reaction vials or plates
- Vortexer

- Pipettes (0.5 to 20 µl, 20 to 200 µl, 100 to 1,000 µl)
- Pipette tips with filters
- Powder-free disposable gloves
- PCR water (nuclease-free)

7. Warnings and precautions for the users

For *in-vitro* diagnostic use.
- 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 instructions for use when 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 (SDS) on R-Biopharm's website.

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 Flu & SARS-CoV-2 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 to the PCR mix of the negative control and of the positive control for each reaction of the Internal Control RNA.

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). Prior to use, thaw the Reaction Mix, the Enzyme Mix, the Positive Control, the No Template Control, and the Internal Control RNA, mix thoroughly (except for the enzyme mix), and centrifuge for a short time. Always cool reagents appropriately during the work steps (2 °C to 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 preparation of the master mix for 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	Enzyme Mix	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 by pipette 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 RT-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 Annealing/Extension	10 sec, 95 °C 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 ABI 7500 Fast Dx, Rotor-Gene Q, CFX96™ Dx and qTOWER³

Reverse transcription	10 min, 58 °C
Initial denaturation	1 min, 95 °C
Cycles	45 cycles
PCR Denaturation Annealing/Extension	15 sec, 95 °C 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	Influenza A/B	Green	**-**
	ICR	Yellow	
	SARS-CoV-2 E gene	Orange	
	SARS-CoV-2 RdRp gene	Red	
Roche LightCycler® 480II	Influenza A/B	465/510	**RIDA®GENE Color Compensation Kit IV (PG0004) is required.**
	ICR	533/580	
	SARS-CoV-2 E gene	533/610	
	SARS-CoV-2 RdRp gene	618/660	
ABI 7500 Fast Dx	Influenza A/B	FAM	**Set the ROX passive reference dye to none.**
	ICR	VIC	
	SARS-CoV-2 E gene	ROX	
	SARS-CoV-2 RdRp gene	Cy5	
Bio-Rad CFX96™ Dx	Influenza A/B	FAM	**-**
	ICR	VIC	
	SARS-CoV-2 E gene	ROX	
	SARS-CoV-2 RdRp gene	Cy5	
Qiagen Rotor-Gene Q	Influenza A/B	Green	**The gain settings must be set to 5 (factory default) for all channels.**
	ICR	Yellow	
	SARS-CoV-2 E gene	Orange	
	SARS-CoV-2 RdRp gene	Red	
qTOWER³	Influenza A/B	FAM	**-**
	ICR	VIC	
	SARS-CoV-2 E-Gen	ROX	
	SARS-CoV-2 RdRp-Gen	Cy5	

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 is present in 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 items before repeating the test:

- Expiration date of the reagents used
- Functionality of the equipment being used
- Correct test procedure

The positive control is made up of synthetic RNA fragments and covers at least one detection system of the corresponding detection channel. Therefore not all detection systems used are necessarily covered by one positive control. For an additional quality control, we therefore recommend performing external controls and predetermined positive patient samples at regular intervals.

11. Sample interpretation

The result interpretation is done according to Table 9.

Table 9: Sample interpretation

Detection of				
E gene (SARS-CoV-2)	**RdRp gene (SARS-CoV-2)**	**M gene/ NP1 gene (Influenza A/B)**	**ICR**	**Result**
negative	negative	**positive**	**positive/ negative**	**Influenza A/B detectable**
positive	**positive**	negative	**positive/ negative**	**SARS-CoV-2 detectable**
positive	**positive**	**positive**	**positive/ negative**	**SARS-CoV-2 and influenza A/B detectable**
positive	negative	negative	**positive/ negative**	**SARS-CoV-2 detectable**
negative	**positive**	negative	**positive/ negative**	**SARS-CoV-2 detectable***
negative	negative	negative	**positive**	**Target gene not detectable**
negative	negative	negative	**negative**	**Invalid**

* Potential weak cross-reactivity to SARS-CoV-1

A sample is positive if the sample RNA and the Internal Control RNA show an amplification signal in the detection system.

The E gene segment detected in this test is a SARS-CoV-2-specific fragment. In the BLAST alignment, the detected RdRp gene segment shows a few matches with the corresponding SARS-CoV-1 sequence segment. Amplification cannot be completely ruled out in this case.

The sensitivity of these two fragments in the SARS-CoV-2 samples within the LoD range can vary slightly, which can lead to only one of the two genes testing positive within the LoD range.

A sample is also positive if the sample 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.

A sample is negative if the sample RNA does not show an amplification signal, but an amplification signal is visible 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.

The device detection limit of the RIDA®GENE assay, using the LightCycler® 480II, is > 50 copies/reaction. Samples with a CT value of > 35 are within the detection limit. It should be noted that the detection limit of the RT-PCR depends on the sample matrix, the Cycler and the RNA extraction and can vary accordingly. We therefore recommend that these samples be assessed as inconclusive, examine the individual curves for sigmoidity and requesting a corresponding follow-up sample taking into account the clinical symptoms. The appropriately trained personnel are responsible for assessing these samples.

12. Limitations of the method

1. This test is intended only for human nasal/throat swabs.
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. 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 the RIDA®GENE Flu & SARS-CoV-2 test.
4. 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.
5. A positive test result does not necessarily indicate the presence of viable organisms. A positive result indicates that the corresponding target genes for influenza A (M gene), influenza B (NP1 gene), and SARS-CoV-2 (E gene; RdRp gene) are present.
6. This assay is intended to be performed in compliance with the regulation on good laboratory practice (GLP). Users must follow exactly the manufacturer's instructions when performing the test.
7. Starting at a tested concentration of 3.0%dihydrocodeine shows an inhibitory effect on the detection of Flu A/B.

13. Performance characteristics

13.1 Precision

The precision of the RIDA®GENE Flu & SARS-CoV-2 real-time PCR test was determined for the following levels of consideration.

Intra-assay precision: Determination of five control samples using 20 replicates each on LightCycler® 480II under identical conditions.

Inter-assay precision: Determination of five control samples in duplicate on ten work days performed by different technicians under reproducible conditions.

Inter-lot precision: Testing for intra- and inter-assay precision was carried out using three different lots.

The obtained coefficients of variation of each measurement using the RIDA®GENE Flu & SARS-CoV-2 real-time PCR test on LightCycler® 480II were less than 2.5 %.

13.2 Analytical sensitivity

13.2.1 Device detection limit

For determining the device detection limit, 20 replicates of a control sample (each with 50 copies/reaction) were measured on LightCycler® 480II. All replicates were positive.

The device detection limit is therefore 50 copies/reaction.

13.3 Analytical specificity

The RIDA®GENE Flu & SARS-CoV-2 real-time PCR test is specific for human SARS-CoV-2 and flu A/flu B in nasal/throat swabs.

Different organisms were also tested. No cross-reactivities with the following species were detected (see Table 10):

Table 10: Cross-reactivity testing

Acinetobacter baumannii strain 5377	-	Adenovirus 1, Human, Adenoid 71 strain	-	Adenovirus 7, Human, strain Gomen	-	*Bordetella parapertussis* strain 12822	-
Bordetella pertussis Tohama 1	-	*Candida albicans*	-	*Chlamydophila pneumoniae*	-	*Corynebacterium diphtheriae*	-
Echovirus 11	-	Epstein-Barr virus strain B95-8	-	*Escherichia coli* (O26:H-)	-	*Escherichia coli* (O6)	-

Escherichia coli (O157:H7)	-	*Enterobacter cloacae*	-	Enterovirus type 71, strain 2003 isolate	-	*Haemophilus influenzae*	-
Herpes simplex virus 1 strain McIntyre	-	Herpes simplex virus 2 strain MS	-	Human coronavirus 229E	-	Human coronavirus OC43	-
Human coronavirus HKU1	-	Human coronavirus NL63	-	Human coxsackievirus A2, strain Fleetwood	-	Human coxsackievirus B4	-
Human cytomegalovirus	-	Human metapneumovirus	-	Human parainfluenza virus 1 strain C35	-	Human parainfluenza virus 2 strain Greer	-
Human parainfluenza virus serotype 3	-	Human parainfluenza virus 4a strain M-25	-	Human parainfluenza virus 4b strain CH19503	-	Human respiratory syncytial virus strain long	-
Human respiratory syncytial virus strain 9320	-	Human rhinovirus genogroup A	-	*Klebsiella pneumoniae* strain MGH 78578	-	*Lactobacillus plantarum*	-
Legionella pneumophila subsp. Pneumophila	-	MERS	-	*Moraxella catarrhalis*	-	Mumps virus genotype G-198	-
Mycoplasma pneumoniae strain FH of Eaton Agent	-	*Neisseria meningitidis* strain FAM18	-	*Pneumocystis jirovecii*	-	*Pseudomonas aeruginosa*	-
Serratia marcescens	-	*Streptococcus pneumoniae* strain NCTC 7465	-	*Streptococcus pyogenes*	-	*Streptococcus salivarius*	-
Staphylococcus aureus	-	*Staphylococcus epidermidis*	-				

13.4 Analytical reactivity

The analytical reactivity of the RIDA®GENE Flu & SARS-CoV-2 multiplex real-time RT-PCR was examined using different strains of influenza A/B viruses and SARS-CoV-2 (see Table 11).

Table 11: Analytical reactivity testing

Strain	Influenza A/B	SARS-CoV-2 (E gene)	SARS-CoV-2 (RdRp gene)
SARS-CoV-2 (isolate: USA-WA1/2020)	negative	positive	positive
SARS-CoV-2 (isolate: Italy-INMI1)	negative	positive	positive
A/Brisbane/02/2018	positive	negative	negative
A/Michigan/45/2015	positive	negative	negative
A/California/7/2009	positive	negative	negative
A/Brisbane/59/2007	positive	negative	negative
A/Guangdong-Maonan/SWL1536/2019	positive	negative	negative
A/Kansas/14/2017	positive	negative	negative
A/Singapore/INFIMH-16-0019/2016	positive	negative	negative
A/Hong Kong/2671/2019	positive	negative	negative
A/Texas/50/2012	positive	negative	negative
A/Perth/16/2009	positive	negative	negative
A/Anhui/1/2013	positive	negative	negative
B/Colorado/06/2017	positive	negative	negative

B/Brisbane/60/2008	positive	negative	negative
B/Washington/02/2019	positive	negative	negative
B/Phuket/3073/2013	positive	negative	negative

13.5 Interfering substances

The presence of RT-PCR inhibitors and interfering substances can lead to false negative or invalid results. Therefore the effects of different substances that could be present in the respective samples because of their widespread use in respiratory tract infections or widespread prevalence (see Table 12) For the substance dihydrocodeine were tested, and an inhibitory effect was observed as of a tested concentration of 3.0% (see Limitations of the method). No interference was identified for the other substances listed (Table 12):

Table 12: List of the substances and concentrations used in the test

Active ingredient/pharmaceutical	Concentration
Ethanol	5% [v/v]
Guanidine hydrochloride	5% [w/v]
Azithromycin	84 mg/mL
Mucins	60 µg/mL
Xylometazoline/nasal spray ratiopharm®	10 % [v/v]
Beclomethasone dipropionate	10 % [v/v]
Paracetamol	10 mg/mL
Amoxicillin	1 mg/mL
Human blood	2 % [v/v]
Sodium chloride	10 % [v/v]
Oseltamivir phosphate	25 mg/mL
Benzydamine hydrochloride	10 % [v/v]

14. Version history

Version number	Section and designation
2020-12-08	Previous version
2021-02-16	2. Summary and explanation of the test 5. Storage instructions 6. Reagents required but not provided 9. Test procedure 10. Quality control 11. Sample interpretation 12. Limitations of the method 13.5 Interfering substances

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Batch number
	Use before
	Store at
REF	Item number
Σ	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Reaction Mix

Enzyme Mix

Internal Control RNA

No Template Control

Positive Control

16. References

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