gb SARS-CoV-2 Influenza A/B

Cat. no.: 3233-100
Cat. no.: 3233-500

CE IVD

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PURPOSE OF USE

gb SARS-CoV-2 Influenza A/B enables the multiplex detection of viral genes of SARS-CoV-2 (Wuhan coronavirus 2019 – E, RdRP genes), Influenza A (M gene) and Influenza B (M gene).

PRINCIPLE OF THE TEST

The test is based on a **one-step RT-qPCR** methodology. The kit contains all the necessary components to perform the test. The kit does not contain reagents to perform the RNA isolation.

INSTRUCTIONS FOR USE

1) Let the reagents thaw completely, mix them thoroughly and spin down briefly prior each use.

Performing RNA isolation

2) Incorporate **10 µl** of **EPC Template RNA** (tube with a yellow cap) into the each isolation reaction.

3) Using **Deionized Water** as a sample with **10 µl** of **EPC Template RNA** in a separate negative isolation control is reccomended.

4) Perform the RNA isolation according to your standard laboratory isolation protocol.

Performing RT-qPCR

5) Pipette **10 µl** (× number of samples) of **Master Mix OneStep Multi** (tube with a blue cap) and **5 µl** (× number of samples) of **Assay CoV-2 Influenza** (tube with a green cap) into the new tube (not included), mix well, spin down, and mark the date of preparation. In this way a **complete assay** is prepared. Handle the mixture according to the instructions in the chapter Storage and manipulation conditions.

   Alternative step: In the case where the **EPC Template RNA** has not been added into the isolation, it can be used as an internal positive control in the PCR by adding **0.25 µl** (× number of samples) into the complete assay.

6) Dispense the prepared complete assay by **15 µl** into micro tubes or plate wells.

7) Add **5 µl** of template to the assay and spin briefly.
   - For each run, an analysis of **Positive Control CoV-2, Positive Control Influenza A, Positive Control Influenza B** and **Deionized Water** as the NTC control is required for proper evaluation.
   - Use RNA prepared according to the steps described above as a template.
   - Analyse RNA as soon as possible after isolation.
   - gb Human B2M mRNA (Cat. no. 3153) kit can be used for the quality control of RNA isolates.

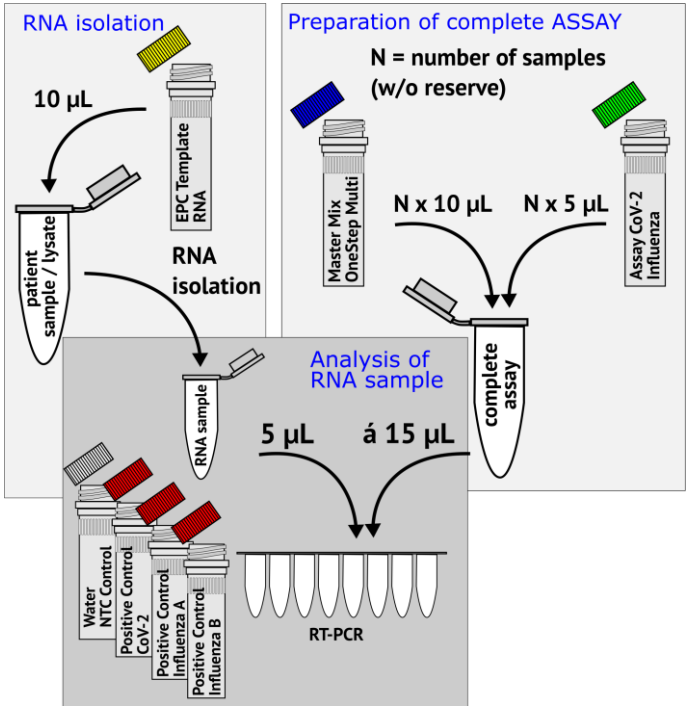
8) Perform sample analysis immediately after reaction mixture preparation.

Amplification protocol and data collection

- Set the PCR cycler to the following temperature profile:

Reverse transcription	42 °C	10 min	
Initial denaturation	95 °C	3 min	
Denaturation	95 °C	10 sec	45 cycles
Annealing + Elongation (+ fluorescence acquisition)	60 °C	30 sec	

- The total volume of a PCR reaction is **20 µl**; please consider this fact when setting up the cycler.
- When using the Rotor-Gene instrument, the **Gain** needs to be set for all channels, so that the basic fluorescence is within **5–10 RFU**. **Attention** – in case of setting the Gain before the analysis, the temperature of Gain optimisation **must not exceed 42 °C**.
- Fluorescence acquisition must be set active for FAM/SYBR, HEX/JOE/VIC, ROX and Cy5 channels.
- Instructions for setting up a cycler can be found at: https://www.generi-biotech.com – see the "download" section, or follow the instrument manufacturer's instructions for use.



DATA ANALYSIS

Determine the SARS-CoV-2, Influenza A or Influenza B presence in a sample by analysing Ct values obtained with the software of your real-time PCR cycler. The limit of detection (LOD) for the kit is 2.2 cop/rxn for SARS-CoV-2, 14.7 cop/rxn for Influenza A and 10.7 cop/rxn for Influenza B.

The LOD has been set at a 95% confidence interval

Read the signal as a Ct (threshold cycle) value in a given fluorescence channel in the quantitation mode. Read the Ct values for **SARS-CoV-2 in FAM,** for **Influenza A in HEX,** and for **Influenza B in ROX channel. The EPC gives signal in Cy5 channel**. For strongly positive samples, the external positive control may not be amplified. For all active channels, **the same threshold fluorescence value (threshold)** must be set for reading. Follow the instructions for use of the cycler manufacturer.

First verify the validity of the analysis and display the signals of control samples.

Analysis validity

The analysis is considered valid when the control signals correspond with the following layout:

- Positive Control CoV-2 – signals in FAM/SYBR and Cy5 channels
- Positive Control Influenza A – signals in HEX/JOE/VIC and Cy5 channels
- Positive Control Influenza B – signals in ROX and Cy5 channels
- Deionized Water as a negative isolation control – a signal in Cy5 channel only
- Deionized Water as NTC – no signal

If the analysis is valid, continue with the evaluation of the samples. Otherwise follow the recommendations given in the Troubleshooting chapter.

Interpretation of results

The final outcome of the analysis is the evaluation of the SARS-CoV-2, Influenza A or Influenza B presence (i.e., sample positivity).

VALID results	**FAM**	**HEX**	**ROX**	**Cy5**
Positive SARS-CoV-2	**+**	**-**	**-**	**+/-**
Positive Influenza A	**-**	**+**	**-**	**+/-**
Positive Influenza B	**-**	**-**	**+**	**+/-**
Negative	**-**	**-**	**-**	**+**
Low positivity	**Ct ⩾ 35**	**Ct ⩾ 35**	**Ct ⩾ 35**	**+**

INVALID results *	**FAM**	**HEX**	**ROX**	**Cy5**
Failed isolation or inhibition of RT-PCR occurred	**-**	**-**	**-**	**-**
Unreliable result	**Ct ⩾ 35**	**Ct ⩾ 35**	**Ct ⩾ 35**	**-**

* gb Human B2M mRNA kit can be used for the quality control of RNA isolates

TROUBLESHOOTING

Test results can be considered correct only if the instructions indicated in the enclosed manual are followed. If the control samples give incorrect results, check the following:

- the expiry date of the kit
- the storage and manipulation conditions
- the pipette and cycler settings

Finding:	**Corrective action suggestion:**
A FAM, HEX and/or ROX signal is detected in the negative control (Deionized Water) reaction.	Reactions were most probably contaminated with a template. Repeat the analysis.
A FAM, HEX and/or ROX signal is repeatedly detected by the same detection assay in the negative control reaction.	The detection assay was most probably contaminated with a template. Repeat the analysis with a new aliquot of the assay.
A FAM, HEX and/or ROX signal is repeatedly detected by all the detection assays in the negative control reaction.	Most probably, Deionized Water was contaminated with a template. Repeat the analysis with a new aliquot of the PCR quality water.
Standard Positive Control was not detected or was detected only in one of the channels.	A pipetting error probably occurred. Repeat the analysis.
A FAM, HEX and/or ROX signal is detected in the negative isolation control; however, no signal was detected in the PCR negative control (NTC).	A cross-contamination between samples probably occurred. Prepare new RNA isolates.
No Cy5 signal is detected in the negative isolation control.	EPC Template RNA was probably not added in the isolation reactions, or a failure of the isolation procedure occurred. Repeat the analysis following the Instructions for use chapter.
No signal in any channel is detected for an examined sample, though the analysis was evaluated as valid.	The inhibition of PCR probably caused the failure of the analysis. Perform the analysis with a new RNA isolate.

CONTENTS AND DESCRIPTION OF KIT COMPONENTS

Component [1]	Volume	Qty [2]	Concentration
Assay CoV-2 Influenza	0.5 ml [3]	1 | 5	4×
Master Mix OneStep Multi	1.0 ml [3]	1 | 5	2×
Positive Control CoV-2	0.2 ml	1 | 1	4×
Positive Control Influenza A	0.2 ml	1 | 1	4×
Positive Control Influenza B	0.2 ml	1 | 1	4×
EPC Template RNA	1.0 ml	1 | 5	
Deionized Water	1.0 ml	1 | 1	

[1] Tube lid colour corresponds with reagent type.
[2] Number for kit size of 100 | 500 reactions.
[3] Volume equates to 100 PCR reactions of 20 µl of volume.

Assay CoV-2 Influenza

Assay CoV-2 Influenza is a mixture of amplification primers and fluorescently labelled probes. The probes enable the detection of viral genes of **SARS-CoV-2 in FAM channel** ($\lambda_{EXCITATION}$ = 495 nm, $\lambda_{EMISSION}$ = 520 nm); **Influenza A in HEX channel** ($\lambda_{EXCITATION}$ = 535 nm, $\lambda_{EMISSION}$ = 556 nm); **Influenza B in ROX channel** ($\lambda_{EXCITATION}$ = 575 nm, $\lambda_{EMISSION}$ = 602 nm) and **external positive control (EPC) in Cy5 channel** ($\lambda_{EXCITATION}$ = 650 nm, $\lambda_{EMISSION}$ = 670 nm). We can therefore detect more signals when the viral genes are present, whereas in their absence only the EPC signal in Cy5 channel is detected. Assay CoV-2 Influenza is supplied in a micro tube with a green cap. Mixing with Master Mix OneStep Multi provides a complete ready-to-use assay.

Master Mix OneStep Multi

Master Mix OneStep supplied in the blue cap micro tube is an optimized mixture of buffer, reverse transcriptase, polymerase and nucleotides that is necessary for RT-qPCR.

Positive Control CoV-2, Influenza A, Influenza B

Positive Control CoV-2/Influenza A/Influenza B serves as a positive control (a standard) for the analysis validity verification. It is supplied in a micro tube with a red cap. Handle Positive Control to avoid cross-contamination with other kit components and analysed samples.

EPC Template RNA

EPC Template RNA is an external positive control for verification of the isolation process. It is supplied in a micro tube with a yellow cap.

Deionized Water

Deionized water serves as a negative isolation control and no-template control (NTC) in PCR. It is supplied in a tube with a transparent lid.

Reagents and equipment not included in the kit

- kit or reagents for the isolation of viral RNA
- single-use plastic micro tubes, strips or plates convenient for use in a PCR cycler
- adjustable micropipettes with the corresponding range
- disposable pipette tips with filters
- laboratory vortex and centrifuge
- real-time PCR cycler with software

WARNINGS AND PRECAUTIONS

Storage and manipulation conditions

- Store all kit components at a temperature below **-20 °C**.
- Assay is photosensitive; limit its handling in the light to the shortest time possible.
- Reagents are designed for work at laboratory temperature.
- Individual kit components may be repeatedly thawed and frozen **5 times at the most**. Do not freeze the complete assay resulting from mixing the Master Mix OneStep Multi and Assay CoV-2 Influenza. The final reaction mixture is **disposable**.
- If the above-mentioned conditions are followed, the kit is stable until its expiry date stated on the **box label**.

Safety measures

- The kit is designed for professional use only.
- When working with RT-qPCR reagents and material, always wear laboratory clothing and safety gloves.
- In case of skin or eye contact with reagents, rinse the affected area under running water.

Instructions for use

- Always use the enclosed version of the manual. The corresponding version number is marked on the label inside the box.
- Inappropriate reagents handling or adjustments of the workflow may negatively influence results and thus it is necessary to strictly follow the pipetting volumes, incubation times and temperature conditions as stated in the manual.
- Adhere to the expiry date of the kit indicated on the box label.
- Do not combine components from different batches of the kit.
- If any of the kit components is damaged upon receipt, do not use it and contact the manufacturer immediately. Keep the component for the purposes of an eventual claim.
- Use calibrated pipettes and instruments.
- Dispose of all waste material in accordance with the applicable legislation. The outer packing is made from paper, the inner segment from polyurethane and the micro tubes from polypropylene. Reagents may be handled as common waste. Dispose of the final PCR analysis product taking into account the risk of work space contamination.

Contamination precautions

- Assign specific spaces, equipment, material and protective equipment for the isolation of RNA/DNA from clinical material, and different ones for preparing RT-qPCR.
- Change your gloves and protective clothing whenever you suspect contamination.
- Never open an amplified PCR product in the place where the PCR reactions are prepared.
- Leave reagents open only for the time necessary to prepare PCR reactions.
- Use tips with filters when pipetting.
- When preparing a reaction mixture, take care not to contaminate any other component of the kit, or other samples, with the positive control. This may be avoided by closing all the micro tubes before manipulating a positive control.
- Use ultra-clean water for sample dilution; the Deionized Water provided with the kit may be used for this purpose.

SYMBOLS USED ON STICKERS

LOT	Batch code
	Expiry date
	Store at recommended temperature
Cont.	Contains
	Manufacturer
	Number of tests

PRODUCT LINE

gb SARS-CoV-2 Multiplex	Cat. no. 3231
gb SARS-CoV-2 Combi E-R	Cat. no. 3232
gb Sarbeco E (primary test)	Cat. no. 3227
gb SARS-CoV-2 RdRP (confirmation test)	Cat. no. 3228
gb Sarbeco N (primary test)	Cat. no. 3229
gb SARS-CoV-2 N (confirmation test)	Cat. no. 3230
gb Human B2M mRNA	Cat. no. 3153

REFERENCES

When using the kit, follow the manufacturer's manual for the cycler. The list of cyclers on which the kit´s performance parameters have been tested is available at the manufacturer's website.

For additional information please contact us at our e-mail address: info@generi-biotech.com or by phone: +420 495 056 314. Further information can also be found on our website www.generi-biotech.com.

GENERI BIOTECH s.r.o.
Machkova 587/42
CZ-500 11, Hradec Kralove - Trebes
CZECH REPUBLIC

www.generi-biotech.com
Phone: +420 495 056 314
E-mail: **info@generi-biotech.com**

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