gb Human B2M mRNA

Cat. no.: 3153-100
3153-500

CE IVD

generi biotech

PURPOSE OF USE

The gb Human B2M mRNA kit enables the detection of human mRNA. Detection is based on the B2M gene (Gene ID 567). This gene encodes beta-2-microglobulin. It is a serum protein found in association with the major histocompatibility complex (MHC) class I heavy chain on the surface of nearly all nucleated cells (excludes red blood cells). The kit is intended to monitor the quality of RNA isolates from human biological material.

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.

INSTRUCTIONS FOR USE

Performing RT-qPCR

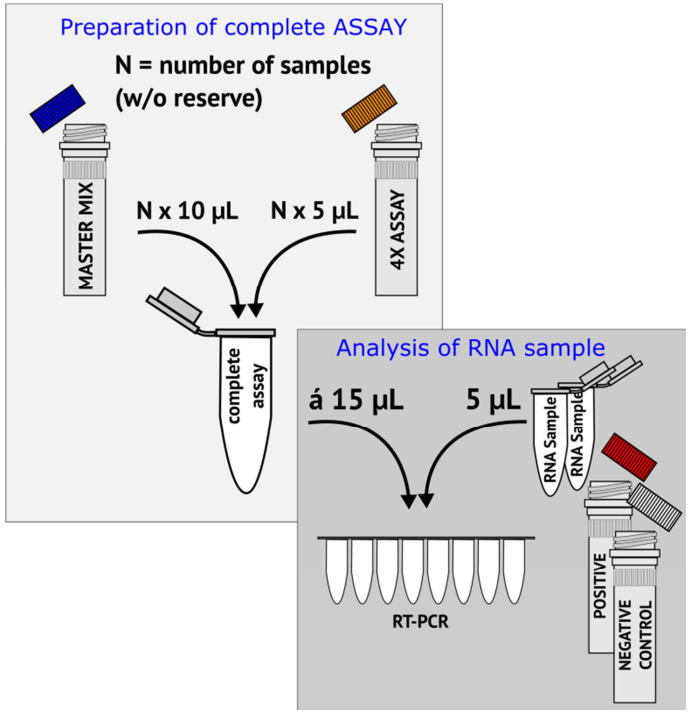
1) Remove the reagents from the box and let them thaw completely.
2) Mix the reagents thoroughly and spin down briefly.
3) Pipette **10 µl** (× number of samples) of **Master Mix OneStep** (tube with blue cap) and **5 µl** (× number of samples) of **Assay hB2M mRNA** (tube with orange cap) into the new tube, mix well, spin down, and mark the date of preparation. In this way a **complete assay** will be prepared Handle the mixture according to the instructions in the chapter Storage and manipulation conditions.
4) Dispense the prepared complete assay with **15 µl** into micro tubes or plate wells.
5) Add **5 µl** of template to the assay and briefly spin.
   - For each run, an analysis of **Standard hB2M** and **Deionized Water** as the NTC control is required for proper evaluation.
   - Use RNA from a biological material as a template.
   - Analyse RNA as soon as possible after isolation.
6) 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	30 min	
Initial denaturation	95 °C	3 min	
Denaturation	95 °C	10 sec	50 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, identical **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 to active for both the FAM/SYBR and HEX/JOE/VIC 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 human mRNA presence in a sample by analysing Ct values obtained with the software of your real-time PCR cycler.

Express the signal as Ct (threshold cycle) in a given fluorescence channel in the quantitation mode. Read Ct values **for target gene in FAM channel, Ct values for internal positive control in HEX channel**. For strongly positive samples, the internal positive control may not be amplified. For both active channels, **the same threshold fluorescence value (threshold)** must be set for reading. Follow the instructions of the cycler manufacturer.

First verify the analysis validity. Display signals of control samples. The analysis validity should be evaluated **for each gene separately**.

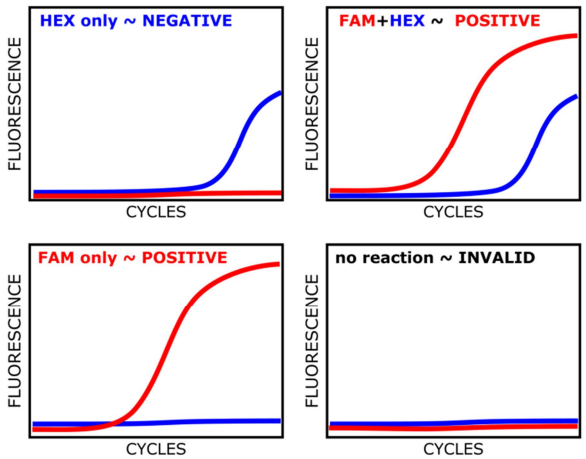
Analysis validity

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

- Standard hB2M – signal in both FAM/SYBR and HEX/JOE/VIC channels
- Deionized Water – signal in HEX/JOE/VIC channel

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

An example of an output of samples – signals in FAM/SYBR (blue curve) and HEX/JOE/VIC (red curve) channels:



Interpretation of results

The basic outcome of the analysis is the evaluation of the human mRNA presence (i.e., sample positivity).

Result	FAM	HEX
Positive	+	+/-
Negative	-	+
invalid (false negative)	-	-

The kit can also be used to monitor the quality of human RNA isolates. **If the analysis is valid but the human B2M gene is not detected (i.e., the result is Negative), low quality and quantity of the RNA isolate can be expected, e.g., due to incorrect sampling of biological material or failure of the isolation procedure.**

A decrease in the efficiency of the biological material collection or recovery yield may not result in a complete absence of the signal, but an increase in the Ct value. The criteria for sample acceptance or exclusion are dependent on the site-specific sample collection practice and isolation procedure used, and their setting is entirely within the competence of the laboratory.

TROUBLESHOOTING

Test results can be considered correct only if the instructions indicated in the enclosed manual are followed. Where 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 signal is detected in the negative control (Deionized Water) reaction.	Reactions were most probably contaminated with a template. Repeat the analysis.
In the negative control reaction, a FAM signal is repeatedly detected by the same detection assay.	The assay was most probably contaminated with a template. Repeat the analysis with a new aliquot of the assay.
In the negative control reaction, a FAM signal is repeatedly detected by all the detection assays.	Most probably, the component Deionized Water was contaminated by a template. Repeat analysis with a new aliquot of the water of PCR quality.
Standard hB2M was not detected or was detected only in one of the channels.	A pipetting error probably occurred. Repeat the analysis.
No signal in FAM and HEX channels was measured for the examined sample, though the analysis was evaluated as valid.	The inhibition of PCR probably caused the failure of analysis. Perform the analysis with a new RNA isolate.

CONTENTS AND DESCRIPTION OF KIT COMPONENTS

Component [1]	Volume	Qty [2]	Concentration
Assay hB2M mRNA	0.5 ml [3]	1 | 5	4×
Master Mix OneStep	1.0 ml [3]	1 | 5	2×
Standard hB2M	0.2 ml	1 | 1	1×10^3 copies /µl
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 hB2M mRNA

Assay hB2M mRNA is a mixture of amplification primers and fluorescently labeled probes. The probes allow the detection and quantification of **target gene in a FAM channel**; $\lambda_{EXCITATION}$ = 495 nm, $\lambda_{EMISSION}$ = 520 nm and **internal positive control in a HEX channel**; $\lambda_{EXCITATION}$ = 535 nm, $\lambda_{EMISSION}$ = 556 nm. We can thus detect two signals when the target gene is present, while in its absence only internal positive control signal in HEX channel will be detected. The Assay is supplied in a micro tube with an orange cap. Mixing with Master Mix OneStep provides a complete ready-to-use assay.

Master Mix OneStep

Master Mix OneStep in the blue cap micro tube is an optimized buffer, polymerase and nucleotide mix that is necessary for RT-qPCR.

Standard hB2M

Standard hB2M serves as positive control for a verification of the analysis validity. 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.

Deionized Water

Deionized water serves as a no-template control (NTC). 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 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; therefore, 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 and Assay hB2M mRNA. 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

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.

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