RT-qPCR Extraction Control
Product Handling Guide

Shipping: On Dry or Blue Ice

Catalog number: MDX028
MDX029

Batch No.: See vial

Concentration: 25x

Store at –80 °C

meridian BIOSCIENCE™

Storage and stability:
RT-qPCR Extraction Control is shipped on dry or blue ice. On arrival store at -80 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:
Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDS will be provided with the first shipment, thereafter they will be available upon request.

Quality control:
Bioline operates under ISO 13485 Quality Management System. RT-qPCR Extraction Control and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

Description

RT-qPCR Extraction Control contains a internal control RNA sequence, with no known homology to any organism, in an artificial cell. The RT-qPCR Extraction Control is spiked with lysis buffer into the sample prior to RNA extraction. Following RNA extraction, the reaction mix is added to the extracted RNA prior to amplification. The presence of internal control RNA confirms the success of the extraction step and reduces the chance of obtaining a false negative result.

Kit components

Table 1

Component
Internal Control RNA
25x Control Mix
50 mM $MgCl_2$

Users Guidelines

Color coding	Internal Control RNA	Control Mix Orange	Control Mix Red	50 mM $MgCl_2$
Cap color	Purple	Yellow	Brown	Blue

Recommended Protocol

All steps should be carried out at room temperature unless otherwise stated. Conditions may vary from reaction to reaction and may need optimisation.

Extraction step

1. Brief spin down all tubes before opening.
2. Standard Protocol:
   i) Spike 2 µL of Internal Control RNA into each sample.
   ii) Follow the manufacturer's protocol for total RNA extraction.
   iii) Elute total RNA in a volume of 100 µL.
3. Use 5 µL of the elution volume for a 20 µL RT-qPCR reaction.
   For example: 2 µL Internal Control RNA spiked into sample, total sample RNA extracted and eluted in 100 µL, 5 µL RNA template is used for a 20 µL reaction.
   Note: This ratio (Internal Control RNA:Elution Vol:RNA template) must be maintained to avoid RNA Extraction Control failure

Post-extraction set up master mix preparation

Recommended reagent volumes per 20 µL RT-qPCR mix are given in Table 2.

Table 2

Component	Supplied	Volume
2x RT-qPCR master mix*	No	10 µL
Target probe/primer mix	No	X µL
Sample RNA from extraction step	No	X µL
25x Control Mix**	Yes	0.8 µL
50 mM $MgCl_2$	Yes	1.2 µL
Reverse transcriptase	No	0.2 µL
RNase inhibitor	No	0.4 µL
Total Volume (for 1 reaction)		20 µL

** This also applies to any commercial RT-qPCR mix with a standard $MgCl_2$ concentration of 3 mM.*
*** Vortex Control Mix before making up the master mix*

Assay setup

The RT-qPCR conditions in Table 3 are suitable for amplicons of up to 200 bp, however they can be varied to suit different commercial RT-qPCR mixes and machine-specific protocols.

Table 3

Step	Temperature	Time	Cycles
Reverse transcription	42 °C	10-20 min	1
Polymerase activation	95 °C	3 min	1
Denaturation	95 °C	10 s	30-40
Annealing/Extension	60 °C	30-45 s	

Acquire RNA Internal Control fluorescence signal on the appropriate channel:
RT-qPCR Extraction Control Red (Quasar 670 - emission wavelength = 670nm)
RT-qPCR Extraction Control Orange (Cal Fluor Orange - emission wavelength = 560nm)

Results

The results can be determined using the following guidelines in Table 4:

Table 4

Results	Target	Internal Control RNA	Interpretation
1	+	+	Target(s) and internal control RNA detected.
2	-	+	Target(s) not detected, internal control RNA detected, indicates a successful extraction and RT-qPCR reaction.
3	-	-	Invalid result: Target(s) and internal control RNA not detected, repeat test.
4	+	-	Invalid result: Internal control not detected, repeat test.

Note:
a) Validation of multiplex PCR should be performed prior to high throughput processes.
b) The negative control reaction should contain all components required for amplification of sample RNA, including Internal Control RNA.
c) A negative control ensures no cross-reactivity with the user-assay and Internal Control RNA.

Technical Support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com

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