RT-qPCR Extraction Control

Catalog numbers

Batch : See vial	MDX028:	500 Rxn	RT-qPCR Extraction Control Red
	MDX029:	500 Rxn	RT-qPCR Extraction Control Orange

Store at –80 °C

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Storage and stability:
RT-qPCR Extraction Control is shipped on dry ice. All kit components should be stored at –80 °C upon receipt. Excessive freeze/thawing is not recommended.

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

Quality Control:
The RT-qPCR Extraction Control is extensively tested for quality and the absence of contamination.

Safety Precautions:
Please refer to the material safety data sheet for further information.

Notes:
For research or further manufacturing use only.

Trademarks:
SensiFAST is a trademark of Bioline Reagents Ltd.

Features

- Easy validation of RNA extraction protocols
- Minimal interference with sample detection
- Includes a ready-to-use reaction mix for easy setup
- Suitable for use with blood, urine and sputum starting samples

Applications

- Monitoring of RNA extraction process in qPCR and RT-qPCR assays

Description

The RT-qPCR Extraction Control enables users of diagnostic assays to validate both their extraction and RT-qPCR. Cells of a known concentration, containing the Internal Control RNA sequence are spiked into the sample tissue and RNA from the sample tissue and the RT-qPCR Extraction Control is simultaneously extracted.

Signal derived from the Internal Control RNA confirms the success of the extraction step and, as a known concentration of cells are added, RT-qPCR Extraction Control also monitors co-purification of PCR inhibitors that may cause biased or false amplification patterns.

Components

Reagent	500 Reactions
Internal Control RNA	5 x 200 µL
Control Mix	5 x 100 µL
50 mM $MgCl_2$	1 x 1.2 mL

Recommended Protocol

Color coding	Internal Control RNA	Control Mix 560	Control Mix 670	50 mM $MgCl_2$
Cap Colors	Purple	Yellow	Brown	Blue

All steps should be carried out at room temperature unless otherwise stated. Conditions may vary depending on the assay and may need optimization.

Extraction step

1. Briefly spin down all tubes before opening.
2. Standard Protocol:
   i) Spike 2 µL of RT-qPCR Extraction Control (REC) 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 PCR reaction.
   For example: 2 µL REC spiked into sample, Total sample RNA extracted and eluted in 100 µL, 5 µL RNA template is used for a 20 µL reaction volume.
   Note: This ratio (REC:Elution Vol:RNA template) must be maintained to avoid RNA Extraction Control failure

Post-extraction setup and analysis

The following RT-qPCR setup is recommended when the REC is to be used with the following:
SensiFAST Probe One-Step No-ROX Kit
SensiFAST Probe One-Step Lo-ROX Kit
SensiFAST Probe One-Step Hi-ROX Kit

1. RT-qPCR set up for SensiFAST Probe One-Step No-ROX Kit.

Component	Supplied	Volume
2x SensiFAST Probe One-Step No-ROX Mix*	No	10 µL
Target Primer/Probe mix	No	X µL
Extracted RNA template	No	X µL
Control Mix**	Yes	0.8 µL
50 mM $MgCl_2$	Yes	1.2 µL
Reverse transcriptase	No	0.2 µL
RiboSafe 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 tube before making up the master mix.*

2. Recommended reverse transcription and PCR cycling conditions.

Cycles	Temperature	Duration	Notes
1	42 °C	10-20 min	Reverse transcription
1	95 °C	3 min	Activation
30-40	95 °C	10 s	Denaturation
	60 °C†	30 s-45 s	Annealing/Extension/ Acquisition

† *The standard annealing temperature is 60 °C, but may have to be optimized by the user, particularly if using an alternative commercial RT-qPCR mix*

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3. Acquire RNA Internal Control fluorescence signal on the appropriate channel (i.e. RT-qPCR Extraction Control Red (Quasar 670 -emission wavelength = 670 nm), RT-qPCR Extraction Control Orange (Cal Fluor Orange -emission wavelength = 560 nm)[††].

[†] *We recommend that the user performs a validation step to ensure that no cross-reactivity exists between the user's primers and the Internal Control RNA. The likelihood of such cross-reactivity is negligible.*
[††] *Ct of the internal control may vary due to elution volume of nucleic acid, use of master mix, number of multiplex etc.*

The results can be determined using the following guidelines:
Note:

Result	Target	REC	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

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 REC
c) A negative control ensures no cross-reactivity with the user-assay and REC

Troubleshooting

Problem	Possible Cause	Recommendation
Invalid Result or Internal Control failure	Not enough RNA template	The correct proportions are as follows: 2 µL REC per clinical sample and an elution volume of 100 µL. Check that the correct amount of extracted RNA template has been added to the reaction.
	RT-qPCR mix not compatible	The REC system requires extra magnesium, adjust final concentration to 6 mM final.
RT-qPCR failure[†††]	RNA contained an RT inhibitor	Remove inhibitors, such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step.
	Reaction conditions not optimal	Increase the primer annealing step from 30 s up to 45 s. Increase the reverse transcription step from 10 min up to 20 min.
	RNA degraded	Analyze RNA on a denaturing gel to verify integrity. Ensure that all reagents are RNase-free.
Poor specificity in RT-qPCR	Primer dimers	Redesign primers to prevent self-annealing. Set up reactions on ice.
	Genomic DNA contamination	Treat RNA with DNase I and re-purify. If possible, use intron-spanning primers in qPCR.
Significant shift in Ct	Inefficient extraction	Alter extraction protocol

[†††] *Shift in Ct or decrease in the fluorescence level (RFU) in the REC signal compared to the expected Ct or normalized fluorescence level*

Technical Support:

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: mbi.tech@meridianlifescience.com

Associated Products

Product	Pack size	Cat. No.
ISOLATE II RNA Mini Kit	10 Preps	BIO-52071
ISOLATE II RNA Plant Kit	10 Preps	BIO-52076
SensiFAST Probe One-Step Hi-ROX Kit	500 reactions	BIO-77005
SensiFAST Probe One-Step Lo-ROX Kit	500 reactions	BIO-78005
SensiFAST Probe One-Step No-ROX Kit	500 reactions	BIO-76005

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