RNA Extraction Control

Catalog numbers

Batch : See vial			
	BIO-38040:	100 Reaction	RNA Extraction Control 670
	BIO-38041:	500 Reaction	RNA Extraction Control 670
	BIO-38044:	100 Reaction	RNA Extraction Control 560
	BIO-38045:	500 Reaction	RNA Extraction Control 560
	BIO-38048:	100 Reaction	RNA Extraction Control 610
	BIO-38049:	500 Reaction	RNA Extraction Control 610

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Storage and stability:
RNA Extraction Control is shipped on dry/blue 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 RNA 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 use only.

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 real-time PCR assays

Description

The RNA Extraction Control (REC) enables users of real-time PCR assays to validate their extraction step. The REC is an artificial cell that contains the control RNA sequence. The REC is spiked into the sample prior to RNA extraction. Following RNA extraction, the Control Mix is added alongside all the components required for amplification of the sample RNA. Signal derived from the Internal Control RNA confirms the success of the extraction step and can also be used to determine the presence of inhibitors in the real-time RT-PCR reaction.

REC contains a sequence with no significant known homology to any published sequence and should not interfere with the detection of the sample RNA, however we recommend performing a negative control reaction.

Components

Reagent	100 Reactions	500 Reactions
REC	1 x 200 µL	5 x 200 µL
25x Control Mix	1 x 100 µL	5 x 100 µL
50 mM $MgCl_2$	1 x 1.2 mL	1 x 1.2 mL

Recommended Protocol

Color coding	Internal Control RNA	Control Mix 560	Control Mix 610	Control Mix 670	50 mM $MgCl_2$
Cap Colors	Purple	Yellow	Orange	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 RNA 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 real-time RT-PCR 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. Real-Time RT-PCR 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
25x Control Mix**	Yes	1 µL
Reverse transcriptase	Yes	0.2 µL
RiboSafe RNase inhibitor	Yes	0.4 µL
Total Volume (for 1 reaction)		20 µL

* *This also applies to any commercial real-time RT-PCR mix with a standard $MgCl_2$ concentration of 3 mM.*
** *Vortex Control Mix tube before making up the mastermix.*

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 real-time RT-PCR mix*

The results can be determined using the following guidelines:

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 real-time RT-PCR 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 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.
	Real-time RT-PCR mix not compatible	The REC system requires extra magnesium, adjust final concentration to 6 mM final.
Real-time RT-PCR 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 real-time PCR	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 real-time PCR.
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: tech@bioline.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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