RT-qPCR Extraction Control

Quality Assurance

- **Simple:** streamlined protocol for straightforward validation of RNA extraction and determination of RT-qPCR assay inhibition
- **Sensitive:** control assay identifies even small effects on RNA extraction and inhibition of amplification
- **Optimized:** control RNA has a sequence with no known homology to any organism, thereby avoiding detection of sample RNA
- **Specific:** probe-based assay designed specifically for multiplex RT-qPCR
- **Flexible:** ideal for use with a wide range of sample types, including inhibitor-rich sample like blood, urine and sputum

RT-qPCR Extraction Control (REC) enables users of a diagnostic RT-qPCR assay to determine if there are inhibitors in the PCR step and also to validate the success of the extraction step, reducing the chance of obtaining a false negative result in the sample RNA.

A common practice in RT-qPCR is to add a known amount of "spiked" control RNA after RNA extraction. This monitors PCR inhibition but has no value as an extraction control. The ideal situation is to have the test sample and internal control undergo the same processing prior to RT-qPCR (Fig. 1). Bioline has developed the RT-qPCR Extraction Control, which more closely mimics the test sample, as compared to spike controls. Genetic material from the test sample and the REC is simultaneously extracted by common methods, with the extraction control being as sensitive to inhibition and extraction failure as the test sample.

REC cells are of a known concentration, containing the Internal Control RNA sequence. This sequence contains no known homology to any organism and importantly, does not interfere with the detection of sample RNA. The REC cells are spiked into the lysis buffer with the target sample, prior to RNA extraction. Control Mix, which includes primers and probe, is then added to the reaction mix before amplification. Signal derived from the Internal Control RNA confirms the success of the extraction step (Fig. 2). REC also monitors co-purification of PCR inhibitors that may cause biased or false amplification patterns.

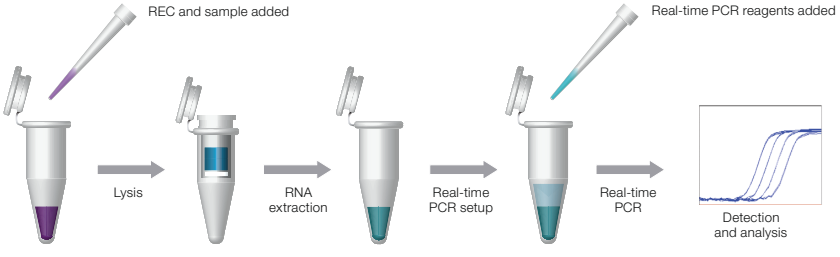

Fig. 1 Overview of the workflow
REC assesses effects of extraction as well as PCR inhibition throughout the entire workflow

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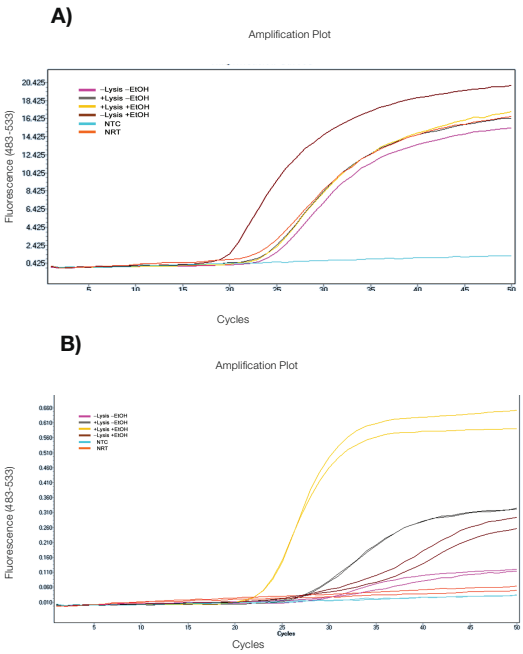

Fig. 2 Inefficient RNA extraction
Control sequences were amplified from A) an internal control DNA and B) REC, ISOLATE II RNA Mini Kit was used (without a DNase step), with the lysis buffer and/or binding buffer being substituted with PBS to stimulate inefficient extraction. The extraction conditions were as follows: Complete lysis (yellow), no lysis (brown), no binding buffer (grey), no lysis and no binding buffer (pink). The results illustrate that the internal control DNA (A) is insensitive to extraction failure as the Ct values remain constant, whereas (B) REC is sensitive as the Ct values decline and so can be used as a control to show the efficiency of the extraction method on the test RNA.

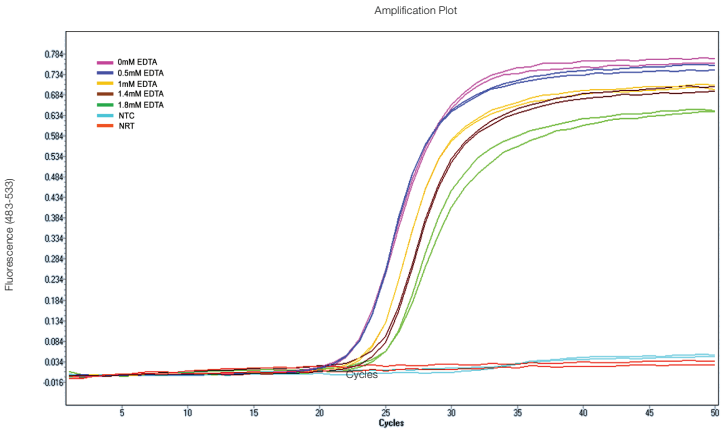

Fig. 3 RT-qPCR reaction inhibition
REC samples were extracted using ISOLATE II RNA Mini Kit. Different concentrations of EDTA were added prior to elution, as an inhibitory agent, to test the monitoring capability of the Internal Control RNA. SensiFAST™ SYBR® One-Step Kit was used with increasing concentrations of EDTA included in the reaction (pink – 0 mM, blue – 0.5 mM, yellow – 1 mM, brown – 1.4 mM and green – 1.8 mM respectively) to simulate increasing concentrations of an inhibitor. The results illustrate that REC is increasingly inhibited by higher concentrations of EDTA, illustrating that inhibition of a RT-qPCR reactions can be identified using REC.

APPLICATIONS

- Pathogen detection
- Cancer risk assessment
- Gene expression analysis
- Drug therapy efficacy
- Biomarker validation
- Copy number variation (CNV) analysis
- Genotyping
- Viral loading

PCR INHIBITION

REC not only serves as an indicator of the effectiveness of the extraction process, but can also be used to monitor co-purification of PCR inhibitors, as REC exhibits higher Ct values relative to the increased concentration of an inhibitory agent (Fig. 3).

Ordering Information

RNA Extraction Controls	Flourescent Dye	Size	Cat. #
RT-qPCR Extraction Control Red	Quasar® 670	500 Reactions	MDX028-1
RT-qPCR Extraction Control Orange	Cal Fluor® Orange 560	500 Reactions	MDX029-1

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