

Real-Time PCR Extraction Controls

- Easy monitoring and validation of DNA and RNA extraction protocols
- Minimal interference with sample detection
- Ideal for blood, urine and sputum samples
- Specially designed for real-time PCR assays

A common practice in real-time PCR is to amplify an internal control DNA after DNA/RNA extraction. Adding internal control DNA after extraction allows for monitoring of inhibition within the assay, but has no value as an extraction control or to show efficiency of reverse transcription. The ideal situation is to have the test sample and internal control undergo the same processing prior to real-time PCR (fig. 1). Bioline has developed a DNA Extraction Control (DEC) and an RNA Extraction Control (REC), which more closely mimic the test sample, as compared to standard internal controls. The genetic material from the test sample and our DEC or REC is simultaneously extracted by common extraction methods, with the extraction control being as sensitive to inhibition and extraction failure as the test sample.

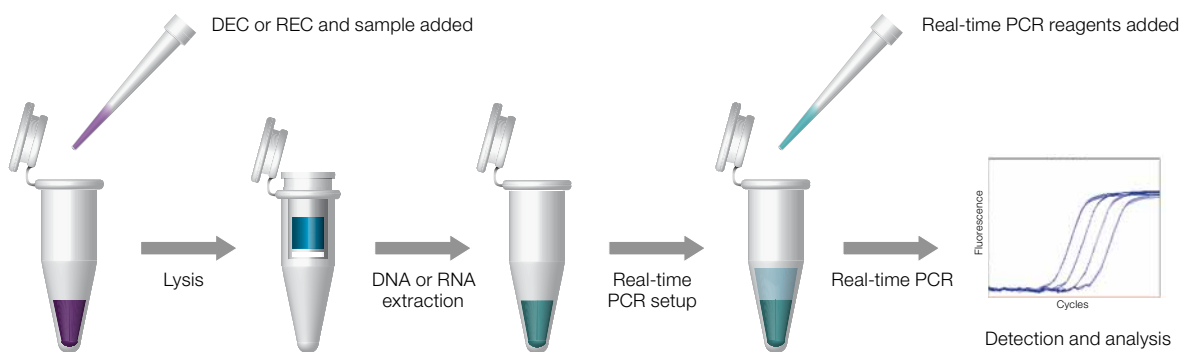


Fig. 1 Overview of the workflow
DEC/REC assesses effects of extraction as well as PCR inhibition throughout the entire workflow.



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DNA Extraction Control

The DEC consists of cells of a known concentration, containing the Internal Control DNA sequence (with no known homology to any organism). These cells are spiked into lysis buffer with the target sample, prior to DNA extraction. Following the extraction, Control Mix (primers and probe) is added to the reaction prior to amplification. Signal derived from the Internal Control DNA creates minimal interference with the detection of the sample DNA and confirms the success of the extraction step (fig. 2).

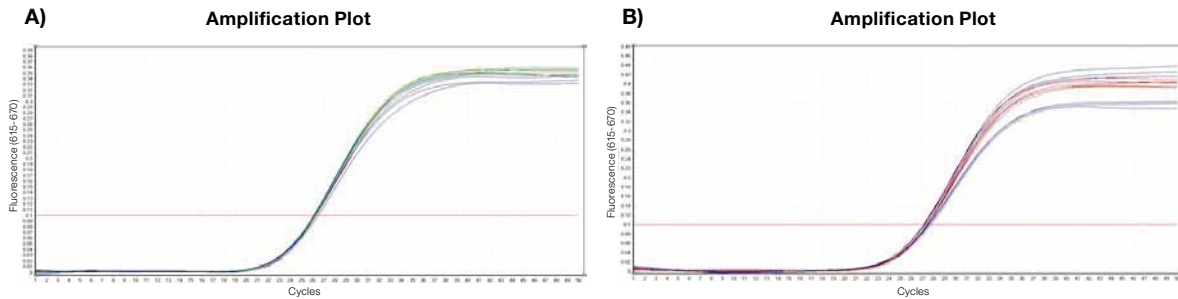


Fig. 2 Minimal interference of DEC in sample detection

A) A fragment of the beta 2 microglobulin (*B2MG*) gene was amplified in triplicate from human genomic DNA in singleplex (green) and in duplex with Internal Control (blue). B) The Internal Control was amplified in singleplex (red) and in duplex with *B2MG* (blue). The Cts show no difference between singleplex (*B2MG* - green, Internal Control - red) and duplex (blue) reaction assays in both target gene and Internal Control.

The DEC 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 the DEC exhibited a similar profile of inhibition to the sample gene, both in Ct and in signal strength (fig. 3).

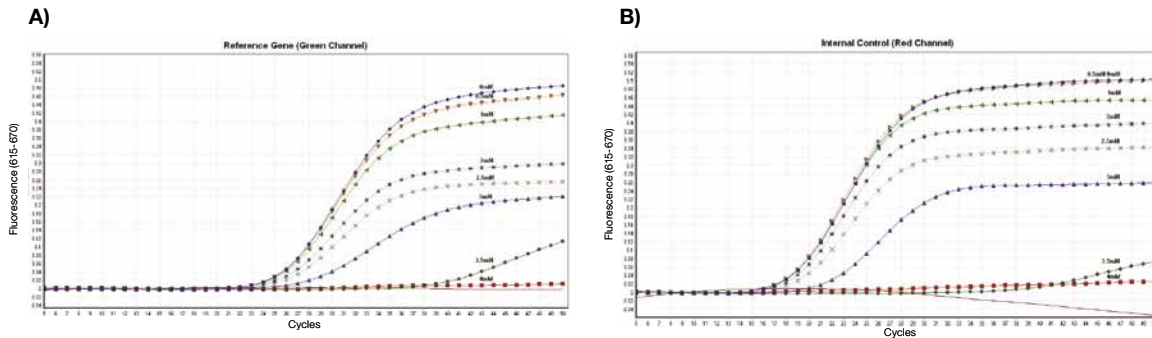


Fig. 3 DEC identifies PCR reaction inhibition

A) A fragment of the beta 2 microglobulin (*B2MG*) gene was amplified from human genomic DNA (green channel) and B) the internal control sequence was amplified from the DEC (red channel). Increasing concentrations of EDTA were included in the reaction (0mM, 1mM, 2mM, 2.5mM, 3mM, 3.5mM and 4mM respectively) to simulate increasing concentrations of an inhibitor. The results illustrate that DEC gives the same pattern of inhibition as with the sample target, showing that inhibition of PCR reaction can be identified using DEC.

The DEC includes viable Alpha Select *E. coli* cells containing the commercially available plasmid pBR322 (genotype: F- deoR endA1 recA1 relA1 gyrA96 hsdR17(r_{k-} , m_{k+}) supE44 thi-1 phoA Δ (lacZYA-argF)U169 Φ 80lacZ Δ M15 λ - pBR322 (Amp r)).



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RNA Extraction Control

The REC consists of artificial cells of a known concentration, containing the Internal Control RNA sequence (with no known homology to any organism). These cells are spiked into lysis buffer with the target sample, prior to RNA extraction. Following the extraction, Control Mix (primers and probe) is added to the reaction mix prior to reverse transcription and amplification. Signal derived from the Internal Control RNA create minimal interference with the detection of the sample RNA and confirms the success of the extraction step (fig. 4).

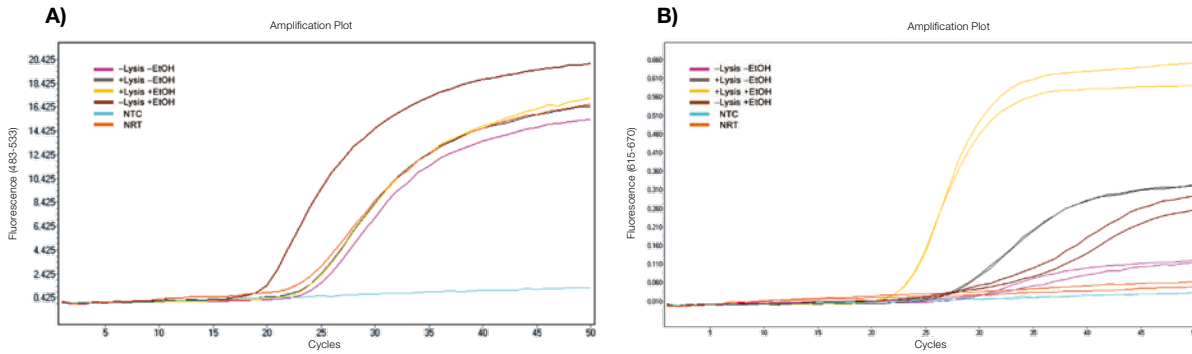


Fig. 4 REC monitors inefficient RNA extraction

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

The 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 illustrated in fig. 5.

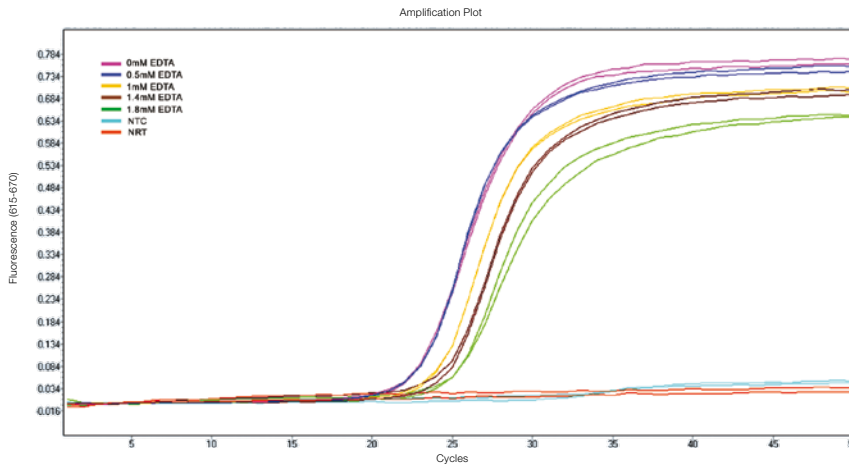


Fig. 5 REC identifies PCR reaction inhibition

REC samples were extracted with a Bioline ISOLATE 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 using SensiFAST SYBR One-Step Kit. Increasing concentrations of EDTA were included in the reaction (pink - 0mM, blue - 0.5mM, yellow - 1mM, brown - 1.4mM and green - 1.8mM respectively) to simulate increasing concentrations of an inhibitor. The results illustrate that REC is increasingly inhibited by increasing concentrations of EDTA, showing that inhibition of PCR reaction can be identified using REC.



Real-Time PCR Extraction Controls

DEC is available with three different dyes (Quasar® 670, Cal Fluor® Orange 560 and Cal Fluor® Red 610) to fit in with existing protocols. CAL Fluor and Quasar dyes are performance-optimized fluorophores for multiplex qPCR.

DEC and REC are suitable for use with commercially available silica-membrane DNA extraction kits and CHELEX matrices and has been tested on a wide range of real-time PCR platforms including ABI-7500, LightCycler 480®, RotorGene-Q™ and MX3005P®.

Please visit www.bioline.com/realtime for more information.

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Trademarks: Quasar® 670, Cal Fluor® Orange 560 and Cal Fluor® Red 610 (Biosearch Technologies), ABI-7500 (ABI), LightCycler 480® (Roche), RotorGene-Q™ (Qiagen) and MX3005P® (Stratagene).

PSGBL0911V1.0

Ordering Information

PRODUCT	FLUORESCENT DYE	PACK SIZE	CAT NO.
DNA Extraction Control 670	Quasar® 670	500 Reactions	BIO-35028
	Quasar® 670	2000 Reactions	BIO-35029
DNA Extraction Control 560	Cal Fluor® Orange 560	500 Reactions	BIO-35031
	Cal Fluor® Orange 560	2000 Reactions	BIO-35032
DNA Extraction Control 610	Cal Fluor® Red 610	500 Reactions	BIO-35033
	Cal Fluor® Red 610	2000 Reactions	BIO-35034
RNA Extraction Control 670	Quasar® 670	100 Reactions	BIO-38040
	Quasar® 670	500 Reactions	BIO-38041
	Quasar® 670	2000 Reactions	BIO-38042
	Quasar® 670	5000 Reactions	BIO-38043



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