

qPCR Extraction Control

Quality Assurance

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

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

A common practice in qPCR is to add a known amount of "spiked" control DNA after sample DNA 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 qPCR (Fig. 1). Bioline has developed the qPCR Extraction Control, which more closely mimics the test sample, as compared to spike controls. Genetic material from the test sample and the DEC is simultaneously isolated with the extraction control being as sensitive to inhibition and extraction failure as the test sample.

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

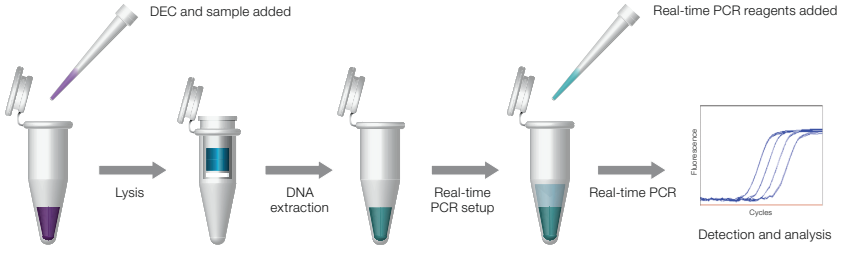


Fig. 1 Overview of the workflow

DEC assesses effects of extraction as well as PCR inhibition throughout the entire workflow.

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APPLICATIONS

- Gene expression analysis
- DNA target detection
- Copy number variation (CNV) analysis
- Pathogen detection
- Cancer risk assessment
- Genotyping
- Viral loading

PCR INHIBITION

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 fluorescent signal strength (Fig. 3).

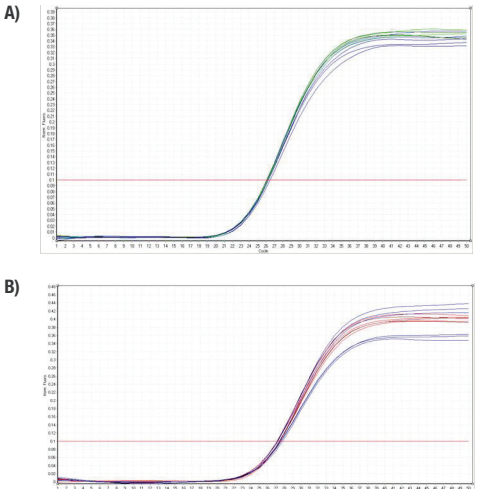


Fig. 2 Minimal interference of DEC in sample detection
DEC was added to human genomic DNA and used in qPCR reactions with SensiFAST™ Probe No-ROX Kit. A) A fragment of the β2-microglobulin (β2MG) gene was amplified in triplicate from the human genomic DNA in singleplex (green) and in duplex with the fragment of Internal Control (blue). B) The results illustrate consistency of Ct values between singleplex and duplex reaction assays and so there is no interference, with both target gene and Internal Control.

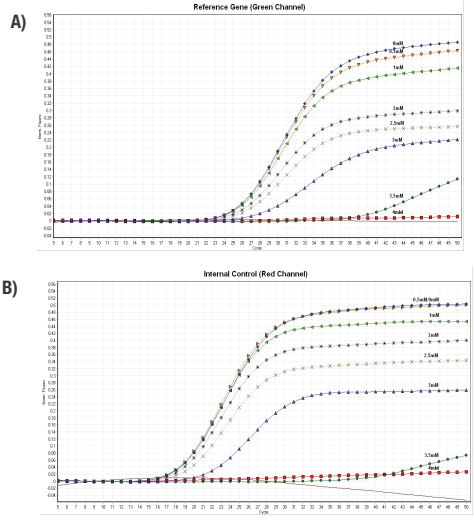


Fig. 3 qPCR reaction inhibition
DEC was added to human genomic DNA and used in qPCR reactions with SensiFAST Probe No-ROX Kit. A) A fragment of the β2-microglobulin (β2MG) gene was amplified from human genomic DNA (green channel) and in the same assay B) the Internal Control DNA was amplified from the DEC (red channel). Increasing concentrations of EDTA were included in the reaction (0 mM, 1 mM, 2 mM, 2.5 mM, 3 mM, 3.5 mM, and 4 mM 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 qPCR reactions can be identified using DEC.

Ordering Information

DNA Extraction Controls	Flourescent Dye	Size	Cat. #
qPCR Extraction Control Red	Quasar® 670	2000 Reactions	MDX026-10
qPCR Extraction Control Orange	Cal Fluor® Orange 560	500 Reactions	MDX027-10

PSGBL0319V3.0

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