qPCR Extraction Control
Product Handling Guide

Shipping:	On Dry or Blue Ice
Catalog number:	MDX026 MDX027
Batch No.:	See vial
Concentration:	25x

Store at –20 °C

meridian BIOSCIENCE™

Storage and stability:
qPCR Extraction Control is shipped on dry or blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

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

Safety precautions:
Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDS will be provided with the first shipment, thereafter they will be available upon request.

Quality control:
Bioline operates under ISO 13485 Quality Management System. qPCR Extraction Control and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

Description

qPCR Extraction Control comprises cells that contain an internal control DNA sequence, with no known homology to any organism, and a specific primer and probe control mix for PCR detection. The cells are added to the sample and lysed and extracted with the sample DNA for downstream amplification by qPCR. The detection of the internal control DNA confirms the success of the extraction step and reduces the chance of obtaining false negative results from the sample DNA.

Kit components

Table 1

Component
Internal Control DNA
25x Control Mix

Users Guidelines

Recommended Protocol

All steps should be carried out at room temperature unless otherwise stated. Conditions may vary from reaction to reaction and may need optimisation.

Extraction step

1. Thaw and brief spin down all tubes before opening.
2. Vortex the internal control tube thoroughly to ensure complete mixing.
3. Add 5 µL of internal control DNA solution per sample to be added to your lysis buffer. For batch extraction, please ensure homogeneity of the lysis buffer/Internal control mixture before loading onto samples for uniform result. The remaining internal control DNA solution can be stored at 4 °C.
4. Follow the manufacturer's protocol for sample DNA extraction.

Post-extraction set up master mix preparation

Recommended reagent volumes per 25 µL qPCR mix are given in Table 2.

Vortex Control Mix before making up the master mix

Table 2

Component	Supplied	Volume
2x PCR master mix	No	12.5 µL
Target probe/primer mix	No	X µL
Sample DNA from extraction step	No	X µL
25x Control Mix (brown cap)	Yes	1 µL
Total Volume (for 1 reaction)		25 µL

Assay setup

The qPCR conditions in Table 3 are suitable for amplicons of up to 200 bp, however they can be varied to suit different commercial qPCR mixes and machine-specific protocols.

Table 3

Step	Temperature	Time	Cycles
Polymerase activation	95 °C	10 min	1
Denaturation	95 °C	15 s	30-40
Annealing/Extension	60 °C	30-60 s	

Acquire DNA Internal Control fluorescence signal on the appropriate channel:
qPCR Extraction Control Red (Quasar 670 - emission wavelength = 670nm)
qPCR Extraction Control Orange (Cal Fluor Orange - emission wavelength = 560nm)

* 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 DNA. 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.

Results

The results can be determined using the following guidelines in Table 4:

Table 4

Results	Target	Internal Control DNA	Interpretation
1	+	+	Target(s) and internal control DNA detected.
2	-	+	Target(s) not detected, internal control DNA detected, indicates a successful extraction and qPCR reaction.
3	-	-	Invalid result: Target(s) and internal control DNA not detected, repeat test.
4	+	-	Invalid result: Internal control not detected, repeat test.

Technical Support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com

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