YOUSEQ

INTERNAL CONTROL DNA

qPCR HANDBOOK

For Research Use Only. Not intended for diagnostic use.

CAT NO.: YSL-qP-INT-DNA-FAM/HEX/ROX/CY5

100 reactions

VERSION 3.0

YOUSEQ

YouSeq Ltd
8 Moorside Place
Moorside Road
Winchester
SO23 7FX
United Kingdom

+44 (0) 333 577 6697
hello@youseq.com

youseq.com

INTENDED USE

This primer/probe mix is designed to be used as an internal extraction control for a qPCR test.It is supplied with an exogenous source of DNA which can be spiked into the extraction lysis buffer and co-extracted with the sample of interest. It is designed to be used by trained professionals in a suitable molecular biology laboratory.

SPECIFICITY

The YouSeq Internal Control qPCR test is designed to specifically detect our unique internal control sequence.

For further information on the detection profile of the product, please do not hesitate to contact our team: support@youseq.com

CONTENTS

Component	Cap Colour	Volume
Internal Control primer/probe mix (specified channel)		100 µL
Internal Control DNA Template		500 µL*
Template Resuspension Buffer (TRB)		1.5 mL

* Supplied lyophilised – requires resuspension. See instructions in resuspension section.

MATERIALS REQUIRED BUT NOT PROVIDED

qPCR MasterMix (Recommended for use in combination with YouSeq Tetra™ 2X qPCR MasterMix range: www.youseq.com).

Nucleic acid template – Internal Control DNA is to be included in the sample extraction. See 'Use of Internal Control DNA' section below.

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, etc.).

qPCR instrument.

BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

General use and set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

Once any reagents are resuspended, mark the tick box on the tube for future reference. After this, or after combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) should be included in every run. To reduce contamination, NTCs and samples can be set up and sealed in a clean lab before moving to the PCR lab.

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BENCH SIDE PROTOCOL

RESUSPENSION

Before first use, resuspend the Internal Control DNA Template with the specified volume of Template Resuspension Buffer, as per the table below:

1. Add the Template Resuspension Buffer and pulse vortex the tube to ensure each is mixed well.

Component	Reagent	Volume	Location
Internal Control DNA Template	TRB	500 µL	Extraction lab

USE OF INTERNAL CONTROL DNA

1. At the relevant step in your extraction protocol, pause and add 5 µL of the Internal Control DNA into the extraction/lysis buffer for each sample that is to be extracted.

Please note: Do not to add this Internal Control DNA directly into the biological sample as this may cause degradation of the control DNA.

2. Continue nucleic acid extraction as per the manufacturer's method.

qPCR REACTION SET UP

When setting up the qPCR reaction mix, the Internal Control primer/probe is designed to be a 20X mix.

Example: 1 µL of Internal Control primer/probe in a 20 µL total reaction volume.

qPCR AMPLIFICATION PROTOCOL

1. Load the plate/tubes onto the qPCR instrument and set up the required qPCR protocol.
    a. Follow the amplification protocol as per the MasterMix manufacturer's instructions.
    b. The primer/probe mix is optimised to work in combination with YouSeq Tetra™ 2X qPCR MasterMix using the protocol in the table below.

Temperature	Time	Number of Cycles
95°C	3 minutes	-
95°C	15 seconds	x 45
60°C 📷	60 seconds	

📷 Collect fluorogenic data through the specified channel during this step.

INTERPRETATION OF RESULTS

This control gives information about the efficiency of the DNA extraction step. The Cq value will vary according to the amount of DNA in the sample. In conjunction with YouSeq Tetra™ 2X qPCR MasterMix, a Cq value of ≤ 31 indicates a successful extraction has taken place. If the signal is later than this, repeating the nucleic acid extraction is advised.

PRODUCT SPECIFICATIONS

Storage

Store at -20C from arrival. The product's shelf life is outlined as an expiry date on the pouch label.

Suitable input material

This product will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is the biggest cause of test failure.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of YouSeq Internal control primer/probe mix is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

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