



YOUSEQ

RNA INTERNAL CONTROL qPCR TEST KIT USER GUIDE

Catalogue No's: YSL-qP-INT-RNA-CY5 / YSL-qP-INT-RNA-HEX
100 reactions

VERSION 1.0

For Research Use Only



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INTENDED USE

This kit is designed to be used as an internal extraction control for a qPCR reaction. The kit is supplied with an exogenous source of RNA which can be spiked into the lysis buffer and co-extracted with the sample of interest. The Internal control is then detected in the Yellow (VIC/HEX) or Blue (Cy5) channel of your qPCR instrument in multiplex with your target of interest. The kit is deliberately primer limited to avoid any risk on inhibiting detection of your target of interest. It is designed to be used by trained users in a suitable molecular biology laboratory environment.

KIT CONTENTS

	Cap Colour	Volume
Internal Control primer/probe (Cy5 or VIC/HEX probe)		110 μ l
Internal Control RNA		500 μ l*
Template resuspension buffer		1.5 ml

* Supplied lyophilised and requires resuspension, see resuspension step below for instructions

RESUSPENSION STEP

Resuspend the designated kit contents with the correct reagent as per the table below. Spin or gently tap the vial to ensure all the contents is at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vial to ensure it is mixed well. Always work with RNA template on ice and follow good laboratory practice to prevent degradation

	Reagent	Volume
Internal Control RNA	Template resus. buffer	500 μ l

MATERIALS REQUIRED BUT NOT PROVIDED

RNA Extraction Kit - This qPCR test kit will work well with high quality RNA derived from any extraction kit with minimal PCR inhibitors present.

Pipettes, micro-centrifuge tubes and general laboratory equipment.

RNA EXTRACTION

Add 5 μ l of the internal extraction control RNA into the extraction/lysis buffer for each sample that you are extracting. Make sure not to add this RNA directly in to your biological sample as this may cause degradation of the control RNA.

Continue RNA extraction as per the manufacturer's method.

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes, and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

Combine the following reagents to create a final test reaction:

Component	Volume
2X qPCR MasterMix	10 μ l
Target primer/probe	X μ l
Internal Control primer/probe mix	1 μ l
Extracted Sample RNA	X μ l
Final Volume	20 μ l

qPCR AMPLIFICATION PROTOCOL

Run the following PCR protocol:

Please note: This YouSeq kit will work with any qPCR instrument capable fluorescence of either Cy5 or HEX (VIC).

	Temperature	Time
RT Step	55°C	10 minutes
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Make sure to collect fluorogenic data through all the required target channels.

INTERPRETATION OF RESULTS

Detection of the Internal control is through the Cy5 or HEX channel depending on the kit version you have chosen. This control gives information about the efficiency of your RNA extraction step. Cq values in the range 28 \pm 3 indicate a good quality extraction has taken place. If your signal is later than this, you may wish to consider repeating the RNA extraction. A high RNA load in the sample can lead to reduced or absent internal extraction control signals.

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20C from arrival. The kit's shelf life is outlined as an expiry date on the pouch label. Once you have prepared the Internal Control, it should be stored frozen and kept on ice whilst in use.

Use good quality RNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality RNA. Good quality is defined as RNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of this Kit is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

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