YOUSEQ

CHO RESIDUAL DNA QUANTITATION KIT USER GUIDE

CAT NO. YSL-qP-CHO-100

100 reactions
With Lyophilised MasterMix

VERSION 5.0

For Research Use Only

YOUSEQ

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INTENDED USE

This YouSeq kit has been designed to quantitate residual DNA from CHO in cell lines which are used in the production of biopharmaceutical products. This is a key impurity for clearance during the purification process. Regulatory guidance for products produced in cell culture makes it very clear that DNA content in the final product should be minimal.

The kit enables quantitative results to be obtained in under 2 hours with sub-picogram levels of sensitivity. The accurate, reliable results allow for high-confidence testing across a broad range of sample applications, including in-process samples and bulk drug substances.

KIT CONTENTS

	Cap Colour	Volume/Rxns
CHO specific primer/probe set (FAM Probe)		110 µl
Positive control template (6.98x10^5 pg DNA/ml)		500 µl*
Lyophilised Tetra 2X qPCR MasterMix		1.1 ml*
MasterMix resuspension buffer		1.5ml
Template resuspension buffer		1.5ml
DNase/RNase free water		1.5ml
ROX passive reference		10 µl

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

RESUSPENSION

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vial to ensure all the contents are at the bottom before opening. After adding the resuspension reagent, pulse vortex the vial to ensure it is mixed well.

	Reagent	Volume
Lyophilised Tetra 2X qPCR MasterMix	MasterMix resus. buffer	1.1 ml
Positive control template (6.98x10^5 pg DNA/ml)	Template resus. buffer	500 µl

MATERIALS REQUIRED BUT NOT PROVIDED

DNA/RNA Extraction Kit – This kit will work well with any DNA extraction kit that yields high quality DNA with minimal PCR inhibitors present.

qPCR instrument.

Pipettes and general laboratory equipment.

ROX (PLATFORM DEPENDENT)

ROX is required for platforms that use ROX as a passive reference. The table below outlines the hardware platforms that require the addition of ROX.

If ROX is required, dilute the ROX supplied according to the table below, then add 5µl to the fully resuspended Tetra MasterMix.

	Instruments	Step 1: Volume of water to add to ROX tube	Step 2: Add to MasterMix vial
High ROX Instruments	Applied Biosystems 7700, 7000, 7900, 7300, StepOne, StepOne Plus, and Roche capillary Lightcyclers 2.0	No Dilution Required	5 µl
Mid ROX Instruments	Stratagene MX	75 µl	5 µl
Low ROX Instruments	Applied Biosystems 7500 Platform, ViiA7 platforms, Quantstudio	130 µl	5 µl
All Other Machines		Not Required	Not Required

qPCR BENCH SIDE PROTOCOL

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

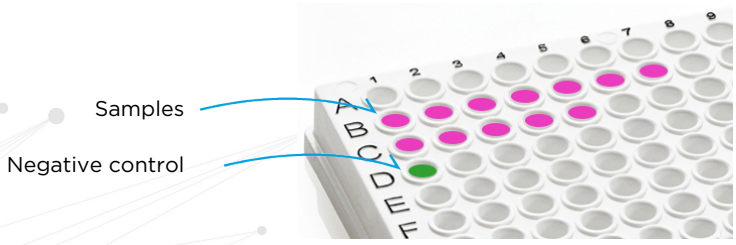
REACTION SET UP

Combine the following reagents to create a test reaction:

Component	Volume
qPCR MasterMix	10 µl
Primer/probe mix	1 µl
Sample DNA	9 µl
Final Volume	20 µl

NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample DNA with DNase/RNase free water.



Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.

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POSITIVE CONTROL STANDARDS

In your designated post-PCR environment, perform a serial dilution of the positive control template to create a six-point standard curve.

1. Add 90µl of template resuspension buffer into 5 tubes and label them 2, 3, 4, 5 and 6
2. Pipette 10 µl of Positive Control Template into tube 2
3. Mix by pipetting up and down 5 times
4. Change pipette tip and pipette 10µl from tube 2 into tube 3
5. Mix by pipetting up and down 5 times

Repeat steps 4 and 5 to complete the dilution process.

Load 6 wells to create your positive control standard curve.

Set up a reaction well for each point of your standard curve using the set-up above but replace the sample DNA with 5µl of standard template plus 3µl of DNase/RNase free water into each well as per the table below:

Tube	Template concentration (pg DNA/reaction)	No. of template copies In PCR reaction (5 µl)
Positive Control (Tube 1)	$3.49x10^{3}$	$1x10^{6}$
Tube 2	$3.49x10^{2}$	$1x10^{5}$
Tube 3	$3.49x10^{1}$	$1x10^{4}$
Tube 4	3.49	$1x10^{3}$
Tube 5	$3.49x10^{-1}$	100
Tube 6	$3.49x10^{-2}$	10

qPCR AMPLIFICATION PROTOCOL

This YouSeq kit will work with any qPCR instrument capable of detecting FAM. Use the following cycling conditions:

	Temperature	Time
	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Data collection for appropriate target channels – FAM

INTERPRETATION OF RESULTS

When analysing Sample Cq values, YouSeq recommends checking the threshold within the run file before interpreting the data. We would suggest setting the threshold to 10% of the relevant positive control End Point Fluorescence (EPF).

Positive Control

Firstly, check the positive control performance. The first point of your standard curve should amplify in a Cq range of approximately 18.5+/-2. Amplification outside of this range suggests a failure and the test should be repeated.

Please note: The positive control in the kit is a representative sequence associated to the designs target region and does not contain the organism's entire genome.

Negative Control

In ideal circumstances, the negative control well should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If this signal is ≥5 Cq values away from your sample signal, then it can be considered negative, and the result is viable.

However, if the negative is <5 Cq away from your sample result then the result is inconclusive and should be repeated.

Positive Samples

Samples that are positive for the target will deliver a defined "sigmoidal" amplification plot.

Quantitation of Results

Use your qPCR instrumentation software to quantify your sample. The assay is designed to have an efficiency of 100%. If the reported efficiency falls outside of the range 90-110% efficiency repeat your dilution series and qPCR for the standard curve wells only.

Contact us at support@youseq.com if you require any further assistance with quantitation calculations.

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20°C from arrival. The qPCR kits shelf life is outlines as an expiry date on the pouch label.
Once you have prepared the positive control it can be stored frozen.

Use good quality DNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality DNA. Good quality is defined as DNA 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 CHO Residual DNA Quantitation Kit is tested against predetermined specifications to ensure consistent product quality. Design of the kit met our robust bioinformatic analysis requirements resulting in a clinically relevant detection profile based on available sequence information. The kit is periodically checked against newly available sequence information to remain clinically relevant.

Technical Assistance

For customer support, please contact:

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

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