YOUSEQ

E.COLI O104:H4 MULTIPLEX qPCR TEST KIT USER GUIDE

CAT NO.: YSL-qPX-IC-E.coli-O104H4-100

100 reactions
With Internal Extraction Control and Lyophilised MasterMix

VERSION 1.0

For Research Use Only

YOUSEQ

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INTENDED USE

The YouSeq® product is a qPCR test kit for detection and quantification of E.coli O104:H4 DNA in good quality nucleic acid samples from a variety of sources. It is designed to be used by trained users in a suitable molecular biology laboratory environment.

KIT CONTENTS

	Cap Colour	Volume
E.coli O104:H4 primer/probe O104 primer/probe (HEX probe) H4 primer/probe (FAM probe) Internal extraction control (Cy5 probe)		110 µl
E.coli O104:H4 Positive control template		500 µl*
Lyophilised Tetra 2X qPCR MasterMix		1.1 ml*
Lyophilised MasterMix resuspension buffer		1.5 ml
Template resuspension buffer		1.5 ml
DNAse/RNase free water		1.5 ml

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

RESUSPENSION STEP

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
E.coli O104:H4 Positive control template	Template resuspension buffer	500 µl
Lyophilised Tetra 2X qPCR MasterMix	MasterMix resuspension buffer	1.1 ml per vial

MATERIALS REQUIRED BUT NOT PROVIDED

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

Pipettes, micro-centrifuge tubes and general laboratory equipment.

This qPCR kit will work on any instrument that detects FAM, HEX and Cy5.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection and quantification of E.coli O104:H4 is designed to have the broadest detection profile possible and detect all clinically relevant strains. The primers and probes have very high (>95%) homology with all reference data within the NCBI database.

If you require more specific data about the detection profile of the kit, please do not hesitate to contact our bioinformatics team: support@youseq.com

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USE OF DNA INTERNAL CONTROL

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

Continue DNA 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
Tetra 2X qPCR MasterMix	10 µl
E.coli O104:H4 primer/probe mix	1 µl
Extracted 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.

POSITIVE CONTROL

For a positive control reaction, repeat the reaction set up above but replace the extracted sample DNA with 9 µL of the resuspended positive control template supplied with the kit.

qPCR AMPLIFICATION PROTOCOL

Run the following PCR protocol:

Please note: If using a qPCR machine that uses ROX as a passive reference, then the passive reference must be turned off or set to "none" indicating no passive reference.

	Temperature	Time
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.

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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. It should amplify in a Cq range of approximately 18.5+/-2 for all targets. If the Cq range is not achieved, this would be a failed test and 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.
The positive control within the kit does not include the internal extraction control sequence. Therefore, the positive control should not be expected to amplify in the internal extraction control channel.

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 later than your sample signal, then it can be considered negative, and the result is viable.

If the negative is <5 Cq later than the sample result, then the result is inconclusive, and the test should be repeated after potential sources of contamination have been removed.

Positive Samples

Samples that are positive for one of the targets will deliver a defined "sigmoidal" amplification plot.

Internal extraction control

If your sample delivers a strong positive result for the target, then the internal extraction control is not required for data interpretation and can be ignored.

If your samples deliver a negative result, then the internal extraction control is useful to interpret the result. The Cq value from the internal extraction control will vary according to the amount of DNA in your sample. A late signal (Cq>28) indicates that only a small amount of host derived DNA was present in your sample. You may wish to repeat sample collection and then repeat the test in order to confirm the negative result.

Results interpretation:

E.coli H4 (FAM Probe)	+	+	-	-	-
E.coli O104 (HEX Probe)	+	-	+	-	-
Internal extraction control (Cy5)	+/-	+/-	+/-	+	-
Result	Positive result for E.coli O104:H4	Positive result for E.coli H4	Positive result for E.coli O104	Negative result	**Failed test** Repeat required

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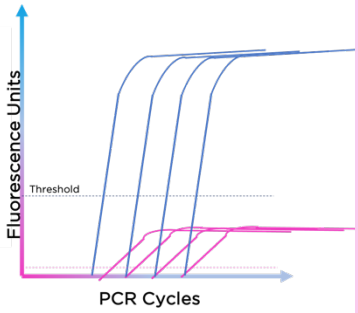
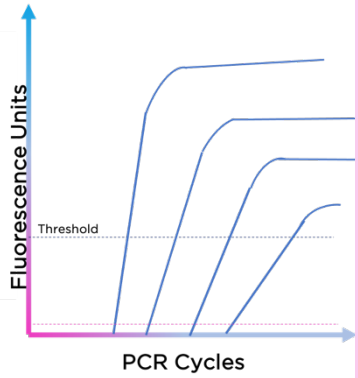
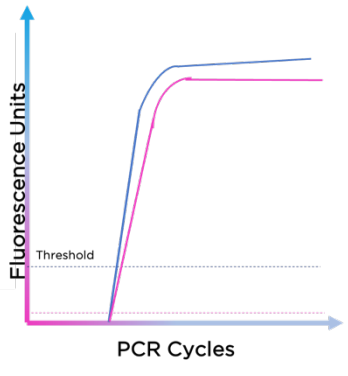
MULTIPLEX TROUBLESHOOTING

	Trace	What can you see?	Cause	Action
1		One assay with greater end point fluorescence than another	Some fluorophores are brighter than others. Also, certain instruments detect different fluorophores with higher/lower efficiency	Analyse each channel individually so the Y-axis is correct for each fluorophore. Or Analyse on logarithmic scale instead of linear scale
2		Traces for weak positives (with later Cq values) appear “leant over” or “flatter” without strong sigmoidal curve	Artefact formation typically driven by Reverse Transcriptase	Minimise the time primer/probe spends in MasterMix during plate set up. Store reagents and set up plate on ice/cold block during experiment set up. Move swiftly to complete plate set up and commence qPCR after plate set-up
3		Amplification/ unusual Cq value in unexpected fluorescent channel. Cq value/curve shape very similar to adjacent fluorescent channel	‘Bleed through’ or ‘cross talk’ between channels. Amplification from one fluorescent channel has been mistakenly identified in its adjacent channel (e.g., FAM identified as HEX)	Ensure manufacturer recommends the dye combination used in this kit. Recalibrate qPCR instrument.

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20C 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 YouSeq E.coli O104:H4 Multiplex 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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