



YOUSEQ

ZIKA, CHIKUNGUNYA & DENGUE MULTIPLEX qPCR TEST KIT HANDBOOK

CAT NO.: YSL-qPX2-EC-DCZ-100

100 reactions
With Endogenous Control and Lyophilised MasterMix

VERSION 8.0

For Research Use Only



YOUSEQ

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INTENDED USE

This product is a qPCR test kit for the detection of Zika, Chikungunya & Dengue (DCZ) RNA 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
Zika virus primer/probe (FAM probe) Chikungunya virus primer/probe (ROX probe) Endogenous control (VIC/HEX probe)		100 µl
Dengue virus primer/probe (FAM probe)		100 µl
DCZ positive control template		500 µl*
Lyophilised Tetra™ OneStep 2X qPCR MasterMix		2 x 1.1 ml*
MasterMix Resuspension Buffer (MMRB)		2 x 1.5 ml
Template Resuspension Buffer (TRB)		1.5 ml
DNase/RNase free water		1.5 ml

* Supplied lyophilised and requires resuspension, 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/tube to ensure all the contents are at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vial/tube to ensure each is mixed well.

	Reagent	Volume
DCZ positive control template	TRB	500 µl
Lyophilised Tetra™ OneStep 2X qPCR MasterMix	MMRB	1.1 ml

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.

qPCR instrument with minimum 3 colour detection (VIC/HEX, ROX and FAM).

Pipettes, micro-centrifuge tubes and general laboratory equipment.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection of Zika, Chikungunya & Dengue (DCZ) 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.

The selected target genes, outlined below, have been demonstrated to have a distinctive sequence within each species making them an ideal target for highly specific detection of these pathogens.

Zika: GP1 gene

Chikungunya: Structural Polyprotein gene

Dengue: sfRNA1 gene

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

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

In a relevant sized sterile container (e.g., Microcentrifuge tube or Falcon tube), combine the following reagents to create enough Reaction Mix for each primer/probe set to cover all the required wells. Mix the combined reagents by briefly vortexing or inverting.

Component	Volume	Volume x N
Tetra™ OneStep 2X qPCR MasterMix	10 µl	10 x N
Zika/Chikungunya OR Dengue specific primer/probe	1 µl	1 x N
Final Volume	11 µl	X µl

Then dispense 11µl of the reaction mix into all the required wells and add 9µl of sample into the relevant wells.

Component	Volume
Reaction Mix (primer/probe & MasterMix)	11 µl
Extracted Sample RNA	9 µl
Final Volume	20 µl

Please note: Work swiftly and on ice. YouSeq Tetra OneStep qRT-PCR MasterMix contains a powerful reverse transcriptase enzyme to deliver maximally efficient conversion of viral RNA to cDNA. This enzyme is active at room temperature. If left at room temperature in the presence of primers/probes the reverse transcriptase can react with the primers and probe to create artefacts that reduce assay sensitivity. Therefore, it is critical to store your primer/probe and MasterMix reaction mix on ice and for periods of no more than 30 minutes.

For the same reason, set up your qPCR reaction plate on ice and proceed to amplification within 30 minutes. Do not delay.

NEGATIVE CONTROL

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

Please note: We advise sealing the sample and negative control wells before proceeding to the post-PCR environment (positive control step).



POSITIVE CONTROL

For a positive control, repeat the reaction set up above replacing the extracted sample RNA 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
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 VIC/HEX, ROX and FAM channels during this step.

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 18.0+/-2 for all targets. If the Cq range is not achieved, this would be a failed test and should be repeated.

Please note: The supplied positive control is a sequence representative of the target region and does not contain the organism's entire genome.

The positive control does not include the control sequence and therefore, should not be expected to amplify in the VIC/HEX 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 the negative control 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, then the result is invalid and the test should be repeated after potential sources of contamination have been removed.

The test is valid if the following conditions are met:

	FAM Channel	ROX Channel
Positive Control	+	+
Negative Control	-	-

Positive Samples

Samples that are positive for the target(s) will deliver a defined “sigmoidal” amplification plot.

Endogenous control

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

If your samples deliver a negative result, then the endogenous control is useful to interpret the result. The Cq value from the endogenous control will vary according to the amount of RNA in your sample. A late signal (Cq>28) indicates that only a small amount of host derived RNA 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 at a glance:

	qPCR Signal				
Zika (FAM probe)	+	-	-	-	-
Chikungunya (ROX probe)	-	+	-	-	-
Dengue (FAM probe)	-	-	+	-	-
Endogenous control (VIC/HEX probe)	+/-	+/-	+/-	+	-

Result

Positive result.
Zika specific RNA detected.

Positive result.
Chikungunya specific RNA detected.

Positive result.
Dengue RNA detected.

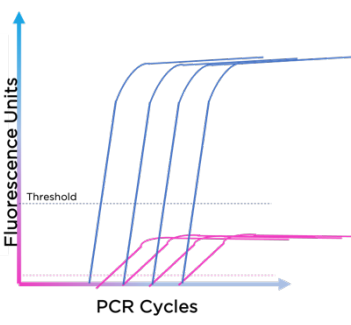
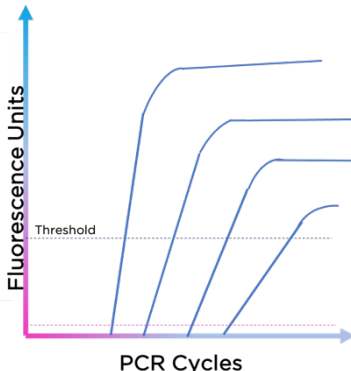
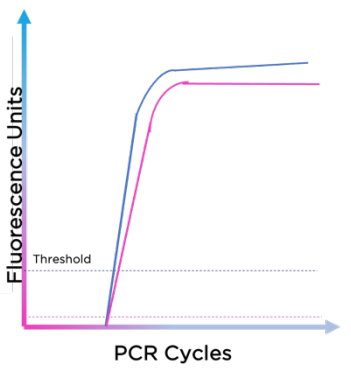
Negative result.

Failed test.
Repeat required

Coinfection

On the rare occasion that a sample contains more than one target pathogen, positive signals in multiple channels will be observed.

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 -20°C from arrival. The qPCR kits shelf life is outlined as an expiry date on the pouch label.

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 YouSeq Zika, Chikungunya and Dengue 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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