ANAPLASMA, BABESIOSIS AND EHRLICHIA MULTIPLEX qPCR TEST KIT USER GUIDE

CAT NO.: YSL-qPX-IC-Ana.Bab.Ehr-100

100 reactions
with Internal Extraction Control and Lyophilised MasterMix

VERSION 3.9

For research use only

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YOUSEQ

INTENDED USE

This product is a qPCR test kit for detection of Babesia SPP (mammalian hosts) (MAMBA) DNA, Ehrlichia SPP (Ehrl. SPP) DNA and Anaplasma SPP (Anap.SPP) 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
MAMBA specific primer/probe (ROX Probe) Ehrl.SPP specific primer/probe (VIC/HEX Probe) Anap.SPP specific primer/probe (FAM Probe) Internal extraction control primer/probe (CY5 Probe)		110 µl
Lyophilised Tetra 2X qPCR MasterMix		1.1 ml*
Ana.Bab.Ehr positive control template		500 µl*
Internal extraction control DNA template		500 µl*
MasterMix resuspension buffer		1.5 ml
DNase/RNase free water		1.5 ml
Template resuspension buffer		1.5 ml

* 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 vials to ensure all the contents is at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vials to ensure it is mixed well.

	Reagent	Volume
Lyophilised Tetra 2X qPCR MasterMix	MasterMix resus. buffer	1.1 ml
Ana.Bab.Ehr positive control template	Template resus. buffer	500 µl
Internal extraction control DNA template	Template resus. buffer	500 µl

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.

qPCR instrument with minimum 4 colour detection (ROX, VIC/HEX, FAM and CY5).

Pipettes, micro centrifuge tubes and general laboratory equipment.

KIT SPECIFICITY

The YouSeq qPCR test kit for detection of Anaplasma, Babesiosis and Ehrlichia (Ana.Bab.Ehr) 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 target genes for Babesia SPP (mammalian hosts) , Ehrlichia SPP and Anaplasma SPP (18s rRNA gene, 16S rRNA gene and 23S gene respectively) have been demonstrated to have unique sequences within these species making them ideal targets for highly specific detection of these pathogens.

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

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.

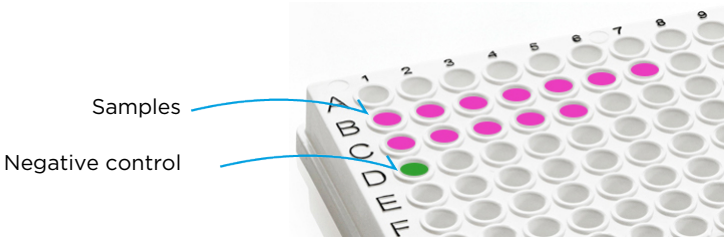
REACTION SET UP

Combine the following reagents to create a final test reaction:

Component	Volume
Tetra 2X qPCR MasterMix	10 µl
Ana.Bab.Ehr specific primer/probe	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 replacing the sample DNA with 9 µl of the 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 ROX, VIC/HEX, FAM and CY5 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. The positive control should amplify in a Cq range of approximately 18.5 +/-2. 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 organisms entire genome.
The positive control does not include the internal control sequence. Therefore, the positive control should not be expected to amplify in the internal control channel.

Negative control

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

The test is valid if the following conditions are met:

	ROX Channel	VIC/HEX Channel	FAM Channel
Positive Control	+	+	+
Negative Control	-	-	-

Positive samples

Samples that are positive for MAMBA, Ehrl.SPP or Anap.SPP will deliver a defined "sigmoidal" amplification plot.

Internal extraction control

If your sample delivers a strong positive result 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.

INTERPRETATION OF RESULTS CONTINUED

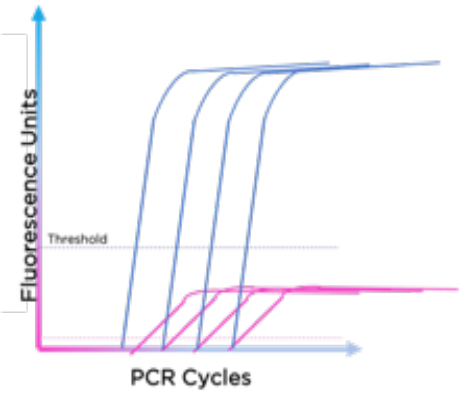
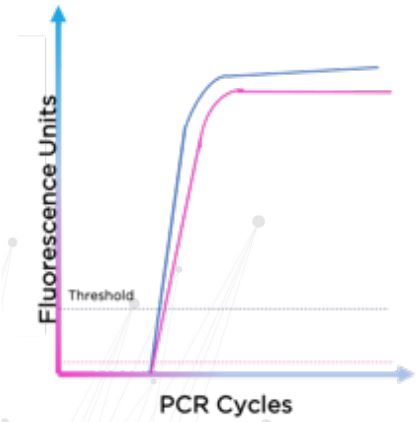
Results interpretation at a glance:

	qPCR Signal				
MAMBA Sample	+	-	-	-	-
Ehrl.SPP Sample	-	+	-	-	-
Anap.SPP Sample	-	-	+	-	-
Internal Extraction Control	+/-	+/-	+/-	+	-
Result	Positive result MAMBA specific DNA detected	Positive result Ehrl.SPP specific DNA detected	Positive result Anap.SPP specific DNA detected	Negative Result	Failed test. Insufficient DNA

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		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 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 Anaplasma, Babesiosis and Ehrlichia Multiplex qPCR 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
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