PNEUMONIA

MULTIPLEX qPCR PANEL HANDBOOK

For Research Use Only. Not intended for diagnostic use.

CAT NO: YS-qPX2-EC-Pneumo-100

100 reactions

VERSION 1.0

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INTENDED USE

This qPCR Panel offers an efficient and user-friendly solution for the detection of the pathogenic markers listed in the **Multiplex Panel Targets** section of this document (see final pages). The primer/probes are to be used in conjunction with good quality nucleic acids extracted from a variety of sample types and include essential control assays to ensure dependable results. It is intended for use by trained professionals in a suitable molecular biology laboratory.

SPECIFICITY AND SENSITIVITY

Specificity

The YouSeq Panel is designed to have the broadest detection profile possible for these pathogens and detect all clinically relevant strains. The primers and probes typically have a ≥95% homology with all reference data used, from relevant, publicly available databases at the time of design.

The target genes, outlined on the final pages, have been demonstrated to have distinctive sequence(s) making them ideal targets for highly specific detection.

For further information on the detection profile of the product, please do not hesitate to contact our team: support@youseq.com

Sensitivity

The Panel is suitable for the detection of these targets across a wide dynamic range. Under ideal PCR conditions the assay can detect less than 100 copies of the target in the PCR reaction.

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CONTENTS

Component	Cap Colour	Volume per tube
Multiplex primer/probe mixes		100 µL
Tetra™ 2X qPCR MasterMix		1 mL
Pneumo Positive Control (PTC)		500 µL*
Template Resuspension Buffer (TRB)		1.5 mL
DNase/RNase Free Water		1.5 mL

* Supplied dried – requires resuspension. See instructions in resuspension section.

RECOMMENDED REAGENTS & MATERIALS

Nucleic Acid extraction kit

General laboratory equipment (pipettes, pipette tips, (micro)centrifuge tubes, compatible strip tubes/plates, plate seals, etc.).

qPCR instrument with channels to detect FAM, VIC/HEX, ROX & CY5.

BEST PRACTICE

Decontamination:

Before beginning laboratory work, thoroughly decontaminate any work surfaces and pipettes being used, to eliminate potential contamination.

General use and set-up:

All components should be fully defrosted with contents at the bottom of the tube before opening. To ensure contents are at the bottom, centrifuge or gently tap the tube. After use, reagents should be returned to the freezer.

After any reagents are resuspended or combined, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the plate and reaction mix on ice to minimise artefact formation, which may reduce sensitivity.

When preparing the qPCR reaction mix, it is recommended to incorporate an overage when calculating the total number of reactions to compensate for potential volume losses incurred during pipetting.

Set-up environments:

It is best practice to set up qPCR plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) for each multiplex should be included in every run. To reduce contamination, NTCs and samples can be set up and sealed in a clean lab before moving to the PCR lab.

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RECOMMENDED BENCH SIDE PROTOCOL

RESUSPENSION

Before first use, resuspend the designated component with the correct reagent and specified volume, as per the table below:

1. Add the resuspension reagent and pulse vortex the tube to ensure each is mixed well.

Component	Reagent	Volume	Location
Pneumo Positive Control (PTC)	TRB	500 µL	PCR lab

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qPCR REACTION SET-UP

Separate reaction mixes are required to be set up for each primer/probe mix.

1. Retrieve the required components and appropriate plasticware for qPCR reaction set-up.

2. In an appropriately sized (micro)centrifuge tube, combine the following reagents to create a reaction mix that will cover all required tube/wells (e.g. samples, NTC and PTC).

Please note: When calculating required reactions, include an overage to allow for volume loss during pipetting

Component	Volume (per reaction)
Tetra™ 2X qPCR MasterMix	10 µL
Multiplex specific primer/probe mix	1 µL
Reaction mix volume	11 µL

3. Mix the combined reagents by briefly vortexing or inverting.

Please note: For best results reaction mixes should be prepared and kept on ice until the amplification protocol is ready to start.

4. Dispense 11 µL of the reaction mix into all required tubes/wells.

5. For the NTC(s), add 9 µL of DNase/RNase Free Water into required tube/well(s).

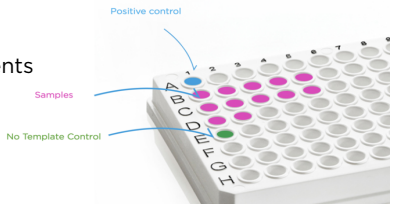
6. For each extracted sample, add 9 µL into required tube/well(s).

Please note: It is best practice to seal the NTC(s) and sample tube/wells before proceeding to the positive control step.

7. For the positive control(s), add 9 µL of the resuspended PTC into the required tube/well(s).

8. Seal the tube(s)/plate.

9. Briefly vortex the sealed tube(s)/plate, then spin in a centrifuge to ensure all reagents are fully resuspended and at the bottom of the tubes/wells before proceeding.



qPCR AMPLIFICATION PROTOCOL

1. Load the tubes/plate onto the qPCR instrument and set up the qPCR protocol following the table below.

2. Set the total reaction volume to 20 µL.

Please note: If using a qPCR instrument that uses ROX as a passive reference, ensure the passive reference is turned off or set to "none" before starting the run.

Temperature	Time	Number of Cycles
95°C	3 minutes	-
95°C	15 seconds	x 40
60°C 📷	60 seconds	

📷 Collect fluorogenic data through FAM, VIC/HEX, ROX and CY5 channels during this step.

3. Start the run.

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INTERPRETATION OF RESULTS – OVERVIEW

If using single threshold analysis – YouSeq recommends setting the threshold at 10% of the End Point Fluorescence (EPF) for each channel.

- For the Target channel, use the PTC EPF to set the threshold.
- For the Endogenous Control, use the average EPF from samples to set the threshold.

Results interpretation:

Reaction Type		qPCR Signal (Cq)	
Positive control:		18 ± 2	18 ± 2
No template control:		-	-
Sample	Endogenous Control:	≤ 28	≤ 28
	Target:	+	-
Result		**Positive result: target specific**	**Negative result**

Coinfection

Positive signals will be observed in multiple channels when a sample contains more than one target pathogen.

INTERPRETATION OF RESULTS – CONTROLS EXPLAINED

Positive control

The PTC should amplify in a Cq range of 18 ± 2 for each target. If this Cq range is not achieved, the sample test result for the associated target is invalid and should be repeated.

Please note: The positive control is a sequence representative of the target regions and does not contain the organism's entire genome. The positive control does not include the Endogenous Control sequence and should not be expected to amplify.

No template control

The NTC well(s) should be negative, with no amplification.

Please note: Background laboratory contamination can result in a very late signal in NTC wells. If the NTC has amplification, comparison to the sample test result is necessary:

- If the NTC is ≥5 Cq later than the sample signal, it can be considered negative, and the sample test result is valid.
- If the NTC is <5 Cq later, the sample test result is invalid – the test should be repeated after potential sources of contamination have been removed.

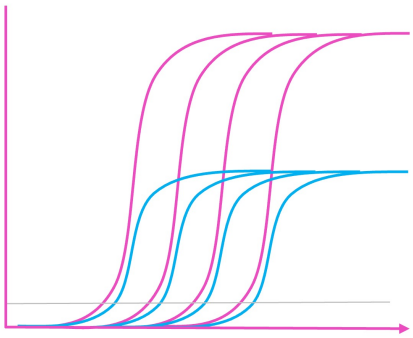
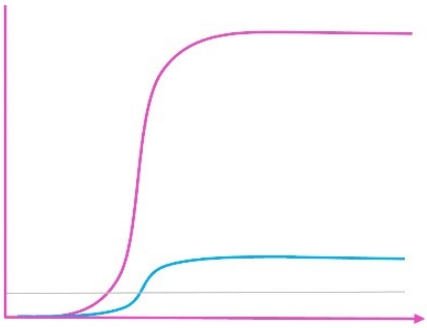
Endogenous Control

The Cq value from the Endogenous Control will vary according to the amount of DNA in the sample. A Cq value of ≤28 indicates a successful extraction has taken place with sufficient host derived DNA in the sample. If the signal is later than this, repeating the nucleic acid extraction is advised.

Please note: If the sample delivers a strong positive result for the target of interest, then amplification of the Endogenous Control may be affected and may appear later. In this case, lack of Endogenous Control amplification is acceptable, and the sample test result is valid.

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MULTIPLEX TROUBLESHOOTING

	1	2
Trace		
What can you see?	Different fluorescence levels between Targets	Unexpected low level of fluorescence, very similar to amplification from a different channel within the same well
Cause	Fluorescence depends on the assay design and the channel e.g. FAM channel is typically the brightest	'Bleed-through' between channels; Amplification from one channel has been incorrectly assigned to a different channel e.g. fluorescence in FAM 'bleeding-through' to VIC/HEX
Action	Analyse each channel individually so the Y-axis is appropriate for that assay	Ensure instrument is compatible with the dye (s) used in this test and/or recalibrate qPCR instrument

PRODUCT SPECIFICATIONS

Storage

Store at -20°C from arrival. The shelf life is outlined as an expiry date on the box label.

Suitable input material

The primer/probe multiplexes will work well with any source of good quality nucleic acid. Good quality is defined as nucleic acid with high integrity (not degraded). Poor quality input nucleic acid is a leading cause of test failure.

Commensal Organisms

Commensal organisms are present as part of a healthy human microbiome and are found on the skin and in the respiratory tract. Contamination of samples and primer/probes is therefore possible, often seen as re-occurring late amplification for any assay targeting a commensal organism.

Regulatory Status

This product has been developed for Research Use Only (RUO) and is not intended for diagnostic use. It should not be used for diagnosis of disease or infection 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 Pneumonia Multiplex qPCR Test Kit is tested against predetermined specifications to ensure consistent product quality. The primers/probes typically demonstrate ≥95% in silico specificity to their intended targets and are periodically checked against newly available sequence information to maintain their detection profile.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
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Internal product identifier
V.0
JSU0313

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MULTIPLEX PANEL TARGETS

MPX	Target	Abbreviation	Target Gene	Potential cross-reactivity	Channel
1	Chlamydia pneumoniae	C.pneum	MOMP gene		FAM
	Mycoplasma pneumoniae	Mp.pneum	internal transcribed spacer (ITS) region		VIC/HEX
	Legionella pneumophila	L.pneum	type IV secretion protein (IcmB) gene	L.cardiaca, L.lytica	ROX
2	Bordetella pertussis	B.pertu	downstream of GAF domain-containing protein gene		ROX
	Bordetella parapertussis	B.parap	downstream of ssb gene		FAM
	RNaseP Endogenous Control	Endo Ctrl	RNaseP gene		VIC/HEX

*Most target genes are detected using an individual assay within the specified channel. Some targets are strategically chosen to be detected in the same channel or have multiple assays to cover all species etc; a positive signal may be from one or more of the target genes.

Potential Cross-Reactivity

Due to extreme homology, it is possible that some of the target sequences may be present and detected in other related species. These potential cross-reactivity scenarios have been outlined in the Panel Targets List.