YOUSEQ

# INFLUENZA A, INFLUENZA B AND RSV

## MULTIPLEX RT-qPCR TEST HANDBOOK

For Research Use Only. Not intended for diagnostic use.

## CAT NO.: YSL-qPX-EC-FluAB.RSV-100

100 reactions
with Endogenous Control and Lyophilised MasterMix

## VERSION 7.6

YOUSEQ

**YouSeq Ltd**
8 Moorside Place
Moorside Road
Winchester
SO23 7FX
United Kingdom

+44 (0) 333 577 6697
hello@youseq.com

youseq.com

YOUSEQ

# INTENDED USE

This RT-qPCR test offers an efficient and user-friendly solution for the detection of Influenza A, Influenza B and RSV in extracted nucleic acid from a variety of sources. It is intended for use by trained professionals in a suitable molecular biology laboratory.

# SPECIFICITY AND SENSITIVITY

## Specificity

The YouSeq RT-qPCR test for detection of Influenza A, Influenza B and RSV is designed to have the broadest detection profile possible 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 below, have been demonstrated to have distinctive sequence making them ideal targets for highly specific detection.
Influenza A (FluA): M1 gene
Influenza B (FluB): NEP/NS1 gene
Respiratory syncytial virus (RSV) (RSV): M gene

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

## Sensitivity

The RT-qPCR test 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.

YOUSEQ

# CONTENTS

| Component | Cap Colour | Volume |
|---|---|---|
| Multiplex primer/probe mix:<br>FluA (FAM )<br>FluB (ROX )<br>RSV (CY5 )<br>Endogenous Control (VIC/HEX ) | | 100 µL |
| Lyophilised Tetra™ OneStep 2X RT-qPCR MasterMix | | 1.1 mL* |
| MasterMix Resuspension Buffer (MMRB) | | 1.5 mL |
| FluAB.RSV 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 ADDITIONAL REAGENTS AND 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, ROX, CY5, and VIC/HEX.

# 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.

Once any reagents are resuspended, mark the tick box on the tube for future reference. After this, or after combining reagents, the tube should be pulse vortexed to ensure it is mixed well.

It is advised to set up the tubes/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 tubes/plates in two different environments - a clean (no template) lab and PCR (template) lab.

No Template Control(s) (NTC) and Positive Control(s) (PTC) 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.

YOUSEQ

# 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 |
|---|---|---|---|
| Lyophilised Tetra™ OneStep 2X RT-qPCR MasterMix | MMRB | 1.1 mL | Clean lab |
| FluAB.RSV Positive Control (PTC) | TRB | 500 µL | PCR lab |

YOUSEQ

## RT-qPCR REACTION SET-UP

1. Retrieve the required components and appropriate plasticware for RT-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™ OneStep 2X RT-qPCR MasterMix | 10 µL |
| FluAB.RSV Multiplex specific primer/probe mix | 1 µL |
| Reaction mix volume | 11 µL |

**Please note:** It is recommended to work quickly with these components, on ice. The activity of Reverse Transcriptase enzymes at room temperature can create artefacts, which may impact assay sensitivity. YouSeq recommends the tube/plate set-up is no longer than 30 minutes.

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

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).

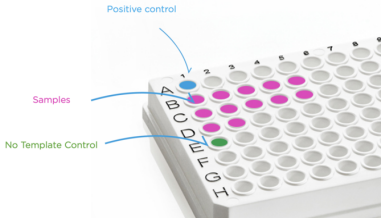
6. For each extracted sample, add 9 µL into required tube/well(s).

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.




## RT-qPCR AMPLIFICATION PROTOCOL

1. Load the tubes/plate onto the qPCR instrument and set up the RT-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 |
|---|---|---|
| 55°C | 10 minutes | - |
| 95°C | 3 minutes | - |
| 95°C | 15 seconds | x 45 |
| 60°C | 60 seconds | |

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

3. Start the run.

YOUSEQ

# 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: (VIC/HEX) | ≤ 28 | ≤ 28 |
| | Targets: (FAM, ROX, CY5) | + | - |
| Result | | **Positive result: target specific**<br>Influenza A (FAM)<br>Influenza B (ROX)<br>Respiratory syncytial virus (RSV) (CY5) | **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 in the VIC/HEX channel.

### 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.

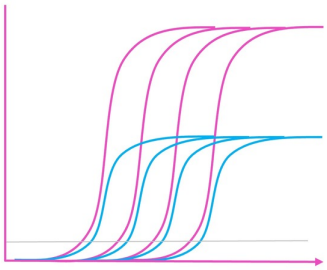
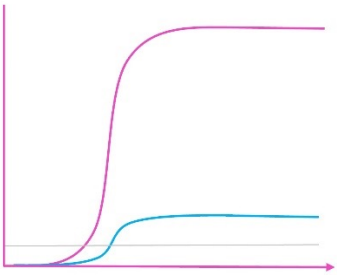
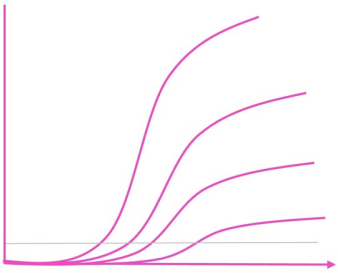
### Endogenous Control

Detection of the Endogenous Control is through the VIC/HEX channel. The Cq value from the Endogenous Control will vary according to the amount of RNA in the sample. A Cq value of ≤28 indicates a successful extraction has taken place with sufficient host derived RNA 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.

YOUSEQ

# MULTIPLEX TROUBLESHOOTING

| | 1 | 2 | 3 |
|---|---|---|---|
| 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 | Amplification for late positives have lower fluorescence, or appear "flatter" than the positive control |
| Cause | Fluorescence depends on the assay design and the channel e. g. FAM channel is typically the brightest | 'Bleed-through' between channels;<br>Amplification from one channel has been incorrectly assigned to a different channel<br>e.g. fluorescence in FAM 'bleeding-through' to VIC/HEX | PCR artefact formation during the run (typically driven by Reverse Transcriptase) |
| 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 | Minimise the time between creating reaction mix (primer/probe + MasterMix) and starting the run.<br><br>Prepare the reaction mix and plate set-up on ice/cold block. |

# PRODUCT SPECIFICATIONS

## Storage

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

## Suitable input material

This RT-qPCR test 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 the leading cause of test failure.

## 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 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 Influenza A, Influenza B and RSV Multiplex RT-qPCR Test Kit is tested against predetermined specifications to ensure consistent product quality. The primers/probe(s) typically demonstrate ≥95% in silico specificity to their intended target 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
phone: +44 (0)333 577 6697

## Trademarks and Disclaimers

YouSeq®, Tetra™, ROX™, VIC™.

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

Not available in all countries.
© 2025 YouSeq Ltd; all rights reserved.