**Kit: HMPV-RPP30**
**Quantity: 100 x 20µL PCR reactions**

**3-plex assay: Human Metapneumovirus (A and B) and human RPP30 mRNA**

**Gene: Matrix Protein**
**SKU#: PNP-HMPV-R-100**

**(RUO). Research Use Only.**
**Not for use in Diagnostic Procedures.**

**Kit contents:**
Tube 1: 5X Primer/Probe mix for HMPVA/B and RPP30 mRNA
Tube 2: 5000 copies/µl Positive controls of synthetic DNA for all targets and hRPP30.

## CONTENTS

The HMPV-RPP30 kit contains a mixture of primers/probes targeting the gene for matrix protein (Protein ID: AHV79911.1) in the HMPVA genome, matrix protein 2.1 (Protein ID: WAB08631.1) in HMPVB genome. The primers and probes in Tube 1 are provided as a 20X concentrated working solution. The fluorophore of the probes for HMPVA and HMPVB is FAM™ and the quencher is BHQ-1™.[1,2] The same mix also contains primers/probe targeting human RPP30 mRNA (20X concentrated) as a RT-PCR positive control assay for human samples. The fluorophore of the RPP30DNA probe is HEX™ and the quencher is BHQ-1™.[2,3] The probes are designed as TaqMan[4] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend RT-qPCR Mastermix, from highQu, Cat number: QOP0405)

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of HMPVA, HMPVB, and RPP30 RNA is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

**Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.**

## KIT HANDLING AND CONTAMINATION

The HMPV-RPP30 kit is shipped at ambient temperature, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed. Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

## EXPERIMENTAL

Set up your reaction (20 µL) as follows on ice:

| Component | Volume (µL) |
|---|---|
| 1Step RT qPCR enzyme mastermix (4X) | 5 |
| RT enzyme (20X) | 1 |
| Primer/Probe mix (20X) | 1 |
| Sample | 2 |
| Water | 11 |

**Note: The composition of this reaction is calculated based on the user manual of RTQAK RT-qPCR MasterMix, from highQu. In a reaction with the double positive control, 2 µL of the solution from Tube 2 should be added.**

A PCR protocol was used in-house for pre-validation on a Bio-Rad CFX96™ Real-Time System, with the following program:

| Step | Thermocycling Protocol: |
|---|---|
| 1 | Incubate @ 50 °C for 10 minutes |
| 2 | Incubate @ 95 °C for 3 minutes |
| 2 | Incubate @ 95 °C for 5 seconds |
| 3 | Incubate @ 55 °C for 15 seconds |
| 4 | Plate Read |
| 5 | Go to Step 3, repeat 44xmore |

## RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 38 cycles and final RFU >200 is considered "positive" or "+" in the Table below.

| HMPV FAM™ | RPP30 HEX™ | Recommended Interpretation |
|---|---|---|
| – | – | The PCR reaction failed. Please repeat the experiment |
| – | + | The sample contains spliced human RPP30 mRNA. The sample doesn't contain HPMV RNA |
| + | – | The sample contains HMPV RNA. The sample may not contain spliced human RPP30 mRNA. |
| + | + | The sample contains HMPV RNA and spliced human RPP30 mRNA. |

## PRE-VALIDATION EXPERIMENTS

The HMPV-RPP30DNA kit validation was carried out as a 3-plex assay, which simultaneously detects RNA from Human Metapneumovirus A, Human Metapneumovirus B, and RNA from the spliced human RPP30 mRNA gene, which serves as a positive extraction-control assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples used for the validation experiments contained $1\times10^4$ copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genomes and the spliced human RPP30 mRNA gene. RNA samples ($\approx5\times10^2$ copies/reaction) extracted from patient samples were also employed, together with total human brain RNA to validate the performance of the kit to detect RNA and DNA targets. The results of these experiments are shown in **Figure 1** below:


**Figure 1:** Validation experiments with single or multiple target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **FAM** probe detects HMPVA/B. The **HEX** probe detects spliced human RPP30 mRNA.

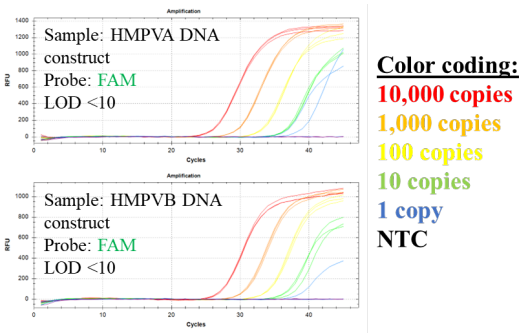


**Figure 2:** Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

**Conclusion:** The data in **Figure 1** indicates that the HMPVA and HMPVB primers and probes are compatible with DNAS RPP30 mRNA positive control primers and probe in a 3-plex application to detect targets in the matrix of human sample extract.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate. For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction for each target.

## CONTACT US

For assistance, please contact DNA Software (the parent company of PCRassays.com) using the link: https://dnasoft.jira.com/servicedesk/customer/portal/1/group/1/create/13

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## NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies, Inc

[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[3] HEX™ (Hexachloro-fluorescein), a trademark of Applera Corp.

[4] "TaqMan" is a trademark of Roche Molecular Systems, Inc.