Kit: RSVB-RPP30
Quantity: 100 x 20µL PCR reactions
Duplexed assay: RSVB with RPP30 control,
Gene: RNA-directed RNA polymerase
SKU: PNP-RSVB-R-100

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

Kit contents:
Tube 1: 20X primers/probe specific for RSVB.
20X primers/probe specific for spliced human RPP30 mRNA.
Tube 2: 5000 copies/µl Positive controls of synthetic DNA for RSVB and hRPP30.
Tube 3: 4X highQu qPCR enzyme mastermix
Tube 4: 20X highQu RT enzyme
(Tubes 3 and 4 are from highQu GmBH).

CONTENTS

The RSVB-RPP30 kit is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) intended for the qualitative detection of nucleic acid from the Human respiratory syncytial virus B (RSVB). The target for the RSVB assay detects the gene for the "RNA-directed RNA polymerase" (Protein ID: QIN86064.1). The primers and probes in Tube 1 are provided as a 20X concentrated working solution. The fluorophore of the probe for RSVB is FAM™ and the quencher is BHQ-1™.[1,2] The same mix also contains primers/probe targeting spliced human RPP30 mRNA (20X concentrated) as a RT-PCR positive control assay for human samples. The fluorophore of the probe is HEX™, and the quencher is BHQ-1™.[3,4]

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of RSVB and hRPP30 is provided as a positive 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**.

Tubes 3 and 4 contain the highQu One-Step RT-qPCR Probe Kit from highQu GmBH (items: QOP0405) are included in this kit appropriate for the TaqMan reaction.[4] This highQu kit provided reproducible and reliable results in pre-validation experiments and is recommended for applications with the RSVB-RPP30 kit. See EXPERIMENTAL section for more details.

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 DNA Software Assay RSVB-RPP30 is shipped with ice packs, 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)
highQu qPCR enzyme mastermix (4X)	5
highQu RT enzyme (20X)	1
RSVB and RPP30RNA primers/probe mix (20X)	1
Sample	2
Water	11

Note: The volume of water should be adjusted accordingly if the user's reaction preparation is different from the recommended preparation method.

An RT-PCR protocol was used at DNA Software, Inc. 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
3	Incubate @ 95 °C for 15 seconds
4	Incubate @ 55 °C for 30 seconds
5	Plate Read
6	Go to Step 3, repeat 44x more
7	(optional) Incubate @55 °C for 3 minutes

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.

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

PRE-VALIDATION DATA

The RSVB-RPP30 kit is a 2-plex assay, where the RSVB assay detects RSVB and RPP30 serves as a control assay to detect human RNA. 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 1×10^4 copies/reaction RNA extract (generously provided by Assurance Scientific Labs) and the total human brain RNA from Roche. The results of these experiments are shown in **Figure 1** below:

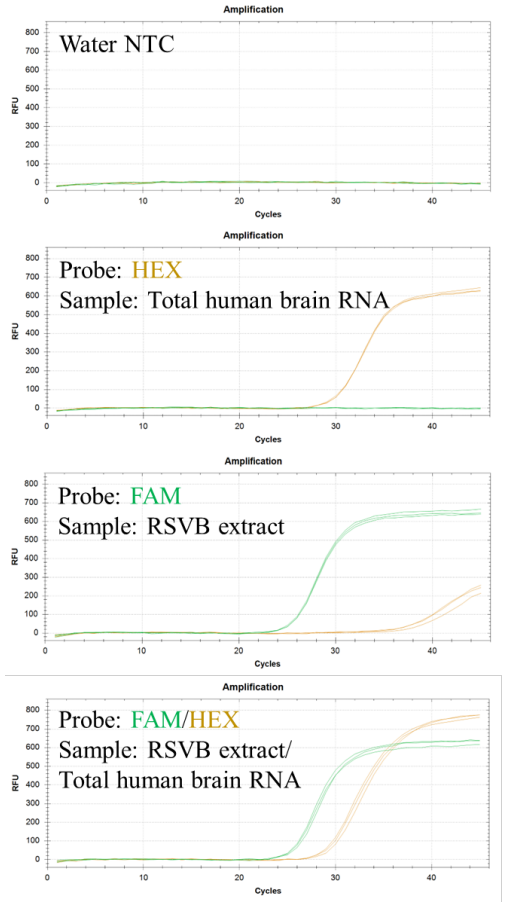

Figure 1: Validation experiments with single target (given in text boxes for each panel) and human mRNA. Both sets of probes and primers are present in every reaction, but positive signal is only observed for one target at a time, indicating that the amplification is specific. The **FAM** probe detects RSVB. The **HEX** probe detects spliced human RPP30 mRNA

CONTACT US

For further assistance, please contact DNA Software using the link: https://www.dnasoftware.com/contact/

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080

NOTES

[1] FAM™ (Carboxyfluorescein) is 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.