Kit: PIV1-RPP30RNA
Quantity: 100 x 20µL PCR reactions
Duplexed assay: Human parainfluenza virus type 1 and human RPP30 RNA
Gene: L polymerase protein
Cat #: PIV1-RPP30RNA-TC-0021

Kit contents:
Tube 1: 20X Primer/Probe mix for PIV1 and hRPP30RNA
Tube 2: 5000 copies/µl Positive controls of synthetic 500 bp DNA for both PIV1 and human RPP30RNA.

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

CONTENTS

The PIV1-RPP30RNA kit contains a mixture of primers/probe targeting the gene of the L polymerase protein (Protein ID: ARB07595.1) in the human parainfluenza virus type 1 (PIV1) genome. The primers and probes in Tube 1 are provided as a 20X concentrated working solution. The fluorophore of the PIV1 probe is CalFluoRed610™ and the quencher is BHQ-2™.[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 RPP30RNA probe is HEX™ and the quencher is BHQ-1™.[3,4] The probes are designed as TaqMan[5] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend QuantiNova Probe RT-PCR kit (100), from Qiagen, Cat number: 208352).

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of PIV1 and human RPP30RNA 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 PIV1-RPP30RNA 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)
Probe Master Mix (2X)	10
RT enzyme (100X)	0.2
Primers/Probe mix (20X)	1
Sample	2
Water	6.8

Note: The composition of this reaction is calculated based on the user manual of QuantiNova Probe RT-PCR kit (100), from Qiagen.

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 @ 45 °C for 10 minutes
2	Incubate @ 95 °C for 5 minutes
3	Incubate @ 95 °C for 5 seconds
4	Incubate @ 60 °C for 30 seconds
5	Plate Read
6	Go to Step 2, repeat 44× more
7	(optional) Incubate 60 °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.

PIV1 (CalFluorRed610™)	RPP30RNA (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment
–	+	The sample doesn't contain PIV1 RNA
+	–	The sample contains PIV1 RNA. The sample may not contain spliced human RPP30 mRNA
+	+	The sample contains PIV1 RNA and spliced human RPP30 mRNA

PRE-VALIDATION EXPERIMENTS

The PIV1-RPP30RNA kit validation was carried out as a duplexed assay, which simultaneously detects RNA from PIV1 and spliced human RPP30 mRNA, 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 1×10^4 copies/reaction of extracted PIV1 (Assurance Scientific Labs) and 100X dilution of human total brain RNA (Takara). The results of these experiments are shown in **Figure 1** below:

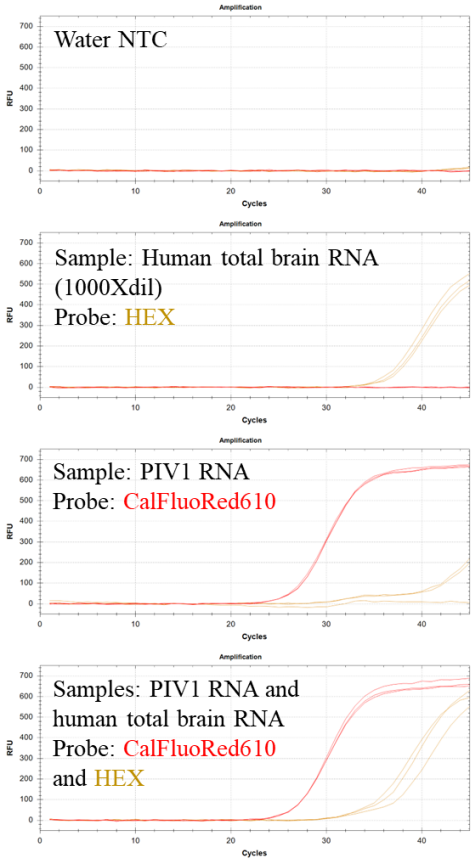
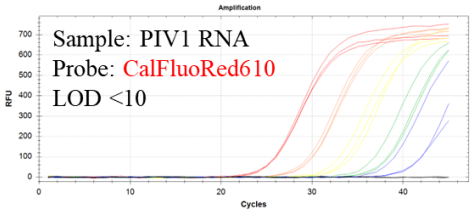


Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **CalFluoRed610** probe detects PIV1 RNA. The **HEX** probe detects spliced human RPP30 mRNA. Due to residual human RNA in extracted PIV1 RNA sample, low amplitude signal from RPP30 RNA is observed in the **HEX** channel in PIV1 RNA samples.



Color coding:
10,000 copies
1,000 copies
100 copies
10 copies
1 copy
NTC

Figure 2: Serial dilution experiments show LOD <10 molecules for PIV1 RNA.

Conclusion: The data in **Figure 1** indicate that the PIV1 primers and probe are compatible with DNAS RPP30RNA positive control primers and probe in a 2-plex application to detect PIV1 in the matrix of human sample extract.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only PIV1 RNA was added. The results show a limit of detection (LOD) <10 copies/reaction.

CONTACT US

For 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] CalFluoRed610™ is a trademark of Biosearch Technologies, Inc

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

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

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

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