PCRassays.com

ASSAY NAME: FLUB_QS

Quantity: 100 x 20µL PCR reactions
2-plex assay: Influenza B and human RPP30 DNA

SKU #: PNP-FLUB-D-QS-100 (QuantStudio)

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

SCOPE OF THIS PRODUCT INFORMATION SHEET:

The oligonucleotide recipes are optimized for each instrument (QuantStudio, BioRad). The verification data presented in this IFU were performed using PNP-VURP5-D-QS-100 on a QuantStudio™ 7 Flex Real-Time System. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you are planning to use a different instrument.

CONTENTS

The primers and probes in the FLUB assay are provided in Tube 1 as a 20X concentrated working solution that detects 1 pathogen and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Flu B	**HEX**	BHQ-1	1,2
RPP30-DNA control	**TAMRA**	BHQ-2	3, 4

The probes are designed as TaqMan[5] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING

The FLUB bundle is shipped on ice, and should be stored at -20 °C. The tubes should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

ASSAY CONTENTS:

Tube 1: 20X Primer/Probe mix for Flu B and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments for Flu B and hRPP30DNA.

Tube 3: InhibiTaq RT-qPCR enzyme Mastermix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

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

Component	Volume (µL)
InhibiTaq RT-qPCR enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

Notes: To improve assay sensitivity, up to 6 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2 (i.e., the "sample"). Molecular biology grade water (NOT included) should be used to prepare the PCR reactions.

A PCR protocol was used for verification on a QuantStudio™ 7 Flex 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 5 seconds
4	Incubate @ 55°C for 22 seconds
5	Plate read
6	Go to step 3, repeat 44x more

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 >200,000 on QuantStudio 5, 6, 7, 12K instruments is considered "positive" or "+" in the Table below.

Flu A HEX™	RPP30 TAMRA™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample contains human RPP30 DNA. The sample doesn't contain bacterial DNA.
+	–	The sample contains Flu B RNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains Flu B RNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The FLUB primers and probes were previously verified as part of the study of a 5-plex assay (SKU#: PNP-VURP5-D-QS-100), which simultaneously detects RNA from Influenza A, SARS-CoV-2, Respiratory syncytial virus, Influenza B, and human RPP30 DNA, which serves as a positive control assay. Data from the Influenza B and control channel are presented below to show the performance expected for the FLUB 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 verification experiments contained 1×10^4 copies/reaction of 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 2-plex specifically detects the pathogen in the human genomic DNA matrix.

NOTES

[1] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Sci.
[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
[3] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.
[4] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
[5] "TaqMan" is a trademark of Roche Molecular Systems, Inc.

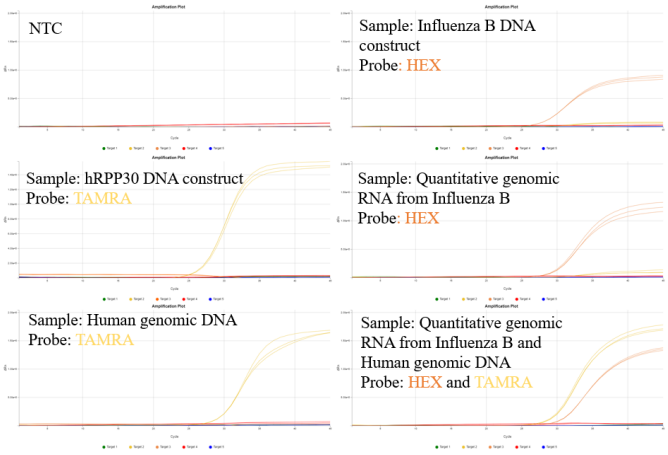

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. Signal from single molecule events show Cq >38 and are considered negative.

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

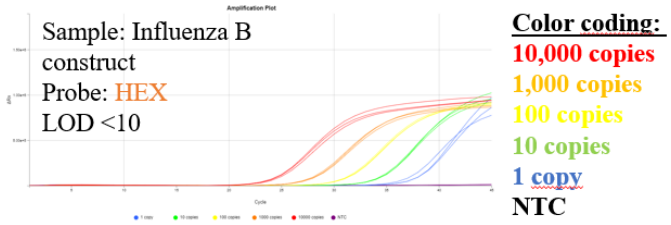

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 2-plex primers and probes are compatible with RPP30_DNA positive control primers.

CONTACT US

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

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