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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Kit: VURP6 (Viral URP 6)

Quantity: 100 x 20µL PCR reactions

4-plex assay: SARS-CoV-2, Influenza A (H3N2 and H1N1), Influenza B, and human RPP30 RNA

SKU: PNP-VURP6-R-QS-100 (QuantStudio)

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

SCOPE OF THIS DOCUMENT:

The oligonucleotide recipes are optimized for QuantStudio. The verification data presented in this PIS were performed using PNP-VURP6-R-QS-100 on a QuantStudio 7 instrument. Contact PCRassays.com if you need to use a different qPCR instrument.

CONTENTS

The primers and probes in the VURP6 kit are provided in Tube 1 as a 5X concentrated working solution that detects 3 pathogens and a human extraction control. It also contains TAMRA used as the passive reference dye during the RT-qPCR reactions.

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
Flu A	FAM	BHQ-1	1,2
RPP30-RNA control	HEX	BHQ-1	6
Passive Reference	TAMRA	none	
SARS-CoV-2	TEX615	BHQ-2	3,4
Flu B	Cy5	BHQ-2	5

The probes are designed as TaqMan[7] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

KIT HANDLING AND CONTAMINATION

The VURP6 kit is shipped at ambient temperature, and should be stored at -20 °C. The kit 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.

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

Kit contents:

Tube 1: Primer/Probe mix (5X, containing TAMRA) for SARS-CoV-2, Flu A, Flu B , and hRPP30RNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of Flu A, SARS-CoV-2, Flu B, and human RPP30RNA.

Tube 3: Spike-in control. 1.0E4 copies/uL of a transcribed human RPP30 mRNA segment.

Tube 4: 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

(Highly recommended) add 1 µL of spike-in control (Tube 3) to each specimen before extraction. **Do not add directly to the PCR reaction!** It serves as extraction and PCR reaction control.

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

Component	Volume (µL)
InhibiTaq 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").

A PCR protocol was used for verification on a QuantStudio 7, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 50°C for 10 minutes
2	Incubate @ 85°C for 1 second
3	Incubate @ 93°C for 2 minutes
4	Incubate @ 93°C for 5 seconds
5	Incubate @ 55°C for 22 seconds
6	Plate read
7	Incubate @ 85°C for 1 second
8	Incubate @ 93°C for 5 seconds
9	Incubate @ 55°C for 22 seconds
10	Plate read
11	Go to step 7, repeat 43x 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.

VERIFICATION EXPERIMENTS

Flu A FAM™	SARS-CoV-2 TEX615™	Flu B Cy5™	RPP30 HEX™	Recommended Interpretation
–	–	-	–	The PCR reaction failed. Please repeat the experiment.
–	–	-	+	The sample contains human RPP30 RNA. The sample doesn't contain viral RNA.
+	–	-	–	The sample contains Flu A RNA. The sample may not contain human RPP30 RNA.
+	–	-	+	The sample contains Flu A RNA and human RPP30 RNA.
–	+	-	–	The sample contains SARS-CoV-2 RNA. The sample may not contain human RPP30 RNA.
–	+	-	+	The sample contains SARS-CoV-2 RNA and human RPP30 RNA.
-	-	+	-	The sample contains Flu B RNA. The sample may not contain human RPP30 RNA.
-	-	+	+	The sample contains Flu B RNA and human RPP30 RNA.
+	+	+	–	The sample contains Flu A RNA, SARS-CoV-2 RNA, and Flu B RNA. The sample may not contain human RPP30 RNA.
+	+	+	+	The sample contains Flu A RNA, SARS-CoV-2 RNA, Flu B RNA, and human RPP30 RNA.

The VURP6 kit verification was carried out as a 4-plex assay, which simultaneously detects RNA from *Influenza A*, *SARS-CoV-2*, *Influenza B*, and human RPP30 RNA, which serves as a positive 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 verification experiments contained 1×10^4 copies/reaction of synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes and human RPP30 RNA gene. RNA standard samples were obtained from ATCC (H1N1 (VR-1894DQ), H3N2 (VR-1882DQ), Flu B (VR-1804DQ)), Twist Biosciences (Twist 50 Omicron BA2), and Takara Bio (human total brain RNA (636530)). **Figure 1** shows the results of these experiments, which indicate that the 4-plex specifically detects the different pathogens.

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.

CONTACT US

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

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

Phone: (734) 222-9080

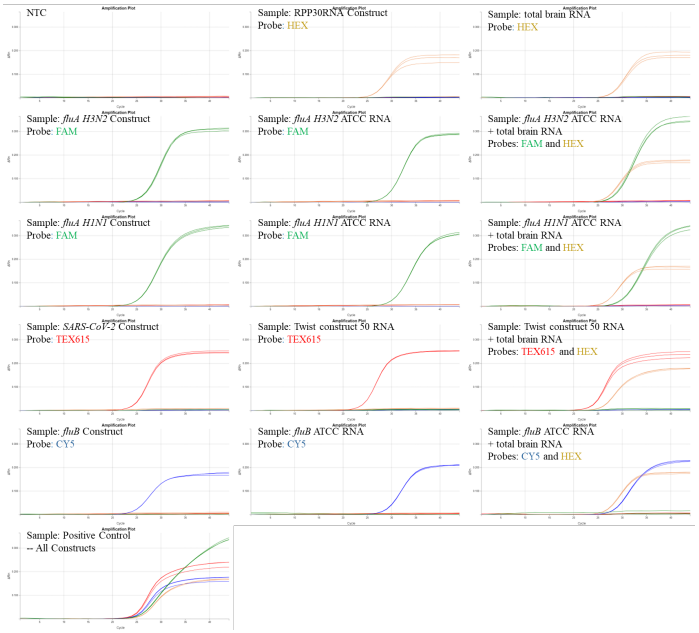

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

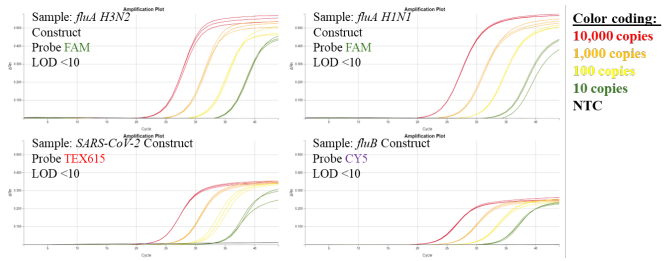

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic RNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 4-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30_RNA positive control primers. Human genomic RNA doesn't interfere with the detection of the pathogens.

NOTES

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

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

[3] TEX615™ is a trademark of Thermo Fisher Scientific.

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

[5] Cy5™, a trademark of GE Healthcare.

[6] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.

[7] TaqMan™ is a trademark of Roche Diagnostics, Inc.