PCRassays.com

ASSAY NAME: ADE2_QS (Adenovirus Panel 2 for QuantStudio)

Quantity: 100 x 20µL PCR reactions

5-color assay Adenovirus types A, D, F40, F41, and human RPP30 DNA

SKU: BUN-ADE2-D-QS-100 (QuantStudio)

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

SCOPE OF THIS PRODUCT INFORMATION SHEET (PIS):

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using BUN-ADE2-D-QS-100 on a QuantStudio™ 7 Flex Real-Time instrument. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you need to use a different qPCR instrument.

CONTENTS

The primers and probes in the ADE2_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects Adenovirus types A, D, F40, and F41. The assay also detects a human extraction control (either endogenous human RPP30-DNA or spike-in RPP30 DNA).

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Adenovirus F40	FAM	BHQ-1	1,2
Adenovirus F41	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4, 5
Adenovirus A	TEX615	BHQ-2	6
Adenovirus D	Cy5	BHQ-2	7

The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING

The ADE2 assay is shipped with frozen cold packs, 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.

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: Primer/Probe mix (5X) for Adenovirus types A, D, F40, F41, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl of DNA constructs of Adenovirus types A, D, F40, F41, and hRPP30DNA.

Tube 3: InhibiTaq Standard 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 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. Molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this assay.

A PCR protocol was used for verification on a QuantStudio™ 7 Flex Real-Time system, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95°C for 2 minutes
2	Incubate @ 95°C for 3 seconds
3	Incubate @ 55°C for 22 seconds
4	Plate Read
5	Go to Step 2, repeat 44× 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.

Adenovirus F40 FAM™	Adenovirus F41 HEX™	hRPP30 TAMRA ™	Adenovirus A TEX615™	Adeno virus D Cy5™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	+	–	–	The sample does not contain DNA of interest. The sample contains human RPP30 DNA.
+	–	–	–	–	The sample contains Ade F40 DNA. The sample may not contain human RPP30 DNA.
+	–	+	–	–	The sample contains Ade F40 DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains Ade F41 DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains Ade F41 DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains Ade A DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	–	The sample contains Ade A DNA and human RPP30 DNA.
–	–	–	–	+	The sample contains Ade D DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains Ade D DNA and human RPP30 DNA.
+	+	–	+	+	The sample contains Ade F40, Ade F41, Ade A, and Ade D DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains Ade F40, Ade F41, Ade A, and Ade D DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The ADE2_QS assay verification was carried out as a 5-plex assay that simultaneously detects RNA from Adenovirus types A, D, F40, F41, and human RPP30 DNA, 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, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different pathogens.

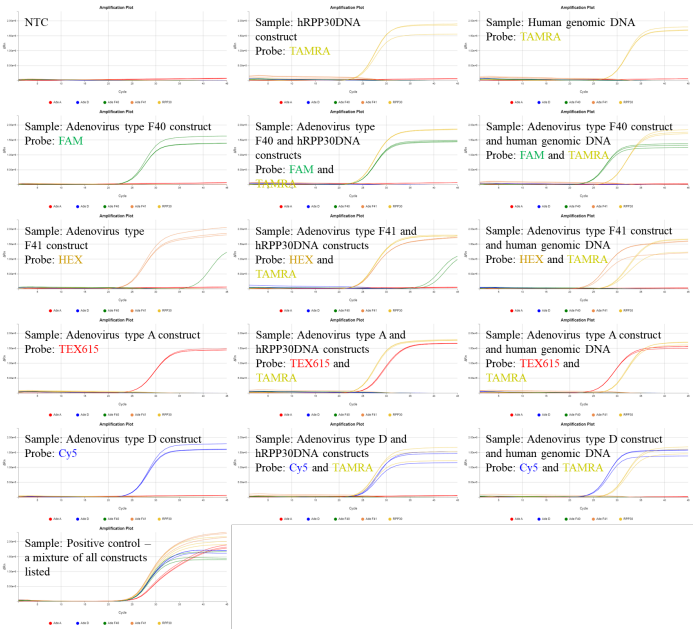

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

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

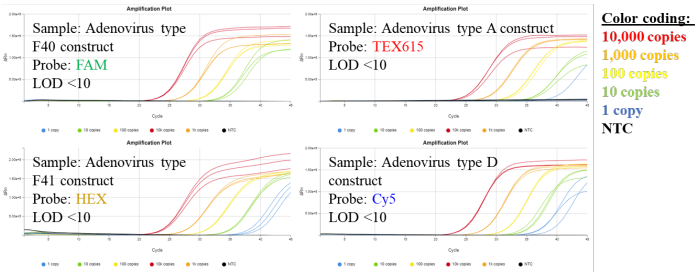

Figure 2: Serial dilution experiments show LOD <10 molecules for the transcribed RNA of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect the pathogens and are also compatible with RPP30 DNA positive control primers.

NOTES

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

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

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

[4] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.

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

[6] CalFluor610™ is a trademark of Biosearch Technologies, Inc.

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

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

CONTACT US

For assistance, please contact DNA Software using the link:
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