PCRassays.com

ASSAY NAME: ADE1_QS (Adenovirus Panel 1 for QuantStudio)

Quantity: 100 x 20µL PCR reactions

5-color assay Adenovirus types B1, B2, C, E, and human RPP30 DNA

SKU: BUN-ADE1-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-ADE1-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 ADE1 assay are provided in Tube 1 as a 5X concentrated working solution that detects Adenovirus types B1, B2, C, and E. 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 B1	FAM	BHQ-1	1,2
Adenovirus E	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4, 5
Adenovirus B2	CalFluor610	BHQ-2	6
Adenovirus C	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 ADE1 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 B1, B2, C, E, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl of DNA constructs of Adenovirus types B1, B2, C, E, 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 > threshold on QuantStudio 5, 6, 7, 12K instruments is considered "positive" or "+" in the Table below. The threshold is typically 200,000 for 20 µL reactions and approximately 20,000 for 10 µL reactions.

Adenovirus B1 FAM™	Adenovirus E HEX™	hRPP30 TAMRA ™	Adenovirus B2 CalFluor610 ™	Adeno virus C 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 B1 DNA. The sample may not contain human RPP30 DNA.
+	–	+	–	–	The sample contains Ade B1 DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains Ade E DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains Ade E DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains Ade B2 DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	–	The sample contains Ade B2 DNA and human RPP30 DNA.
–	–	–	–	+	The sample contains Ade C DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains Ade C DNA and human RPP30 DNA.
+	+	–	+	+	The sample contains Ade B1, Ade E, Ade B2, and Ade C DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains Ade B1, Ade E, Ade B2, and Ade C DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The ADE1 assay verification was carried out as a 5-plex assay that simultaneously detects RNA from Adenovirus types B1, B2, C, E, 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.

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

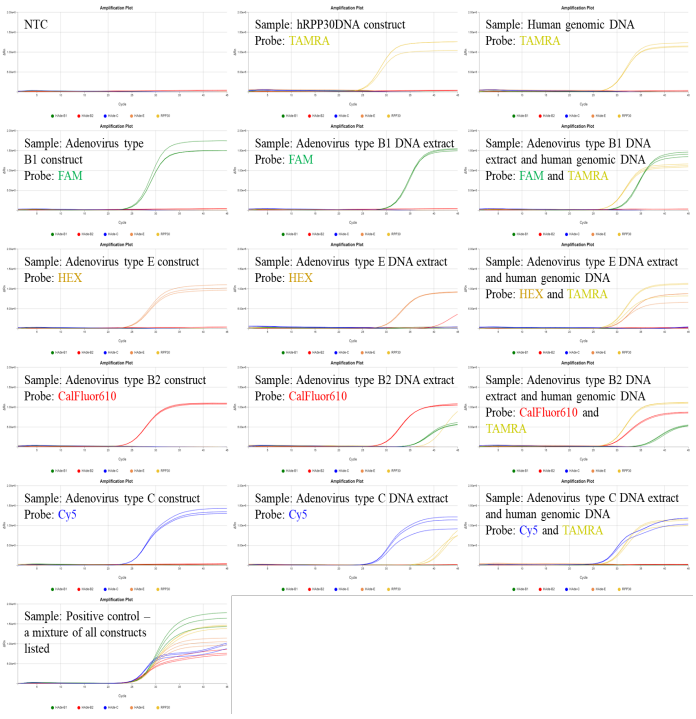

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. Some of the extracts have human DNA present and thus show TAMRA signal from the RPP30 control. The Adenovirus B1 assay (FAM) also detect Adenovirus B2 with late Cq.

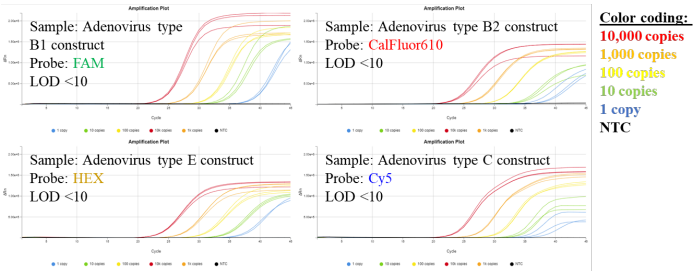

Figure 2: Serial dilution experiments show LOD <10 molecules for the DNA of each target.

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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