PCRassays.com

ASSAY NAME: ADE1 (Adenovirus Panel 1)

Quantity: 100 x 20µL PCR reactions

5-plex assay: Adenovirus B1, Adenovirus B2, Adenovirus C, Adenovirus E, and human RPP30DNA

SKU: PNP-ADE1-D-BR-100 (Bio-Rad)

(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 PNP-ADE1-D-BR-100 on a BioRad CFX96. 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 ADE1 kit contains a mixture of primers/probes for detecting Adenovirus B1 (synonym: mastadenovirus type 7), Adenovirus B2 (synonym: mastadenovirus type 55), Adendovirus C (synonyms: mastadenovirus types 1, 2, 5, 6, 57), and Adenovirus E (synonym: mastadenovirus type 4). The primers and probes in the AdenoP1 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human extraction control. The primers/probes target genes for 100 kDa hexon assembly-associated protein (Protein ID: AYI99503.1) in the HAdV-B1 genome, DNA polymerase (Protein ID: QMV83745.1) in the HAdV-B2 genome, a hypothetical protein (Protein ID: AGT77062.1) in the HAdV-C genome, and hexon protein (Protein ID: ANQ44457.1) in the HAdV-E genome.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
Adenovirus B1	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	7
Adenovirus B2	TEX615	BHQ-2	3,4
Adenovirus C	Cy5	BHQ-2	5
Adenovirus E	Cy5.5	BHQ-2	6

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

Assay contents:

Tube 1: Primer/Probe mix (5X) for Adenovirus B1, Adenovirus B2, Adenovirus C, Adenovirus E, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of Adenovirus B1, Adenovirus B2, Adenovirus C, Adenovirus 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.

ASSAY HANDLING

The ADE1 assay is shipped at ambient temperature, but 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.

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 (NOT included) should be used to prepare the PCR reactions.

A PCR protocol was used for verification on a BioRad CFX96 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 15 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 (BioRad) is considered "positive" or "+" in the Table below.

Adenovirus B1 FAM™	Adenovirus E Cy5.5™	hRPP30 HEX™	Adenovirus B2 TEX615™	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.

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.

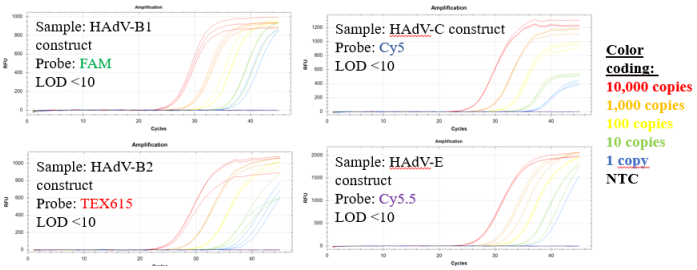

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

VERIFICATION EXPERIMENTS

The ADE1 assay verification was carried out as a 5-plex assay that simultaneously detects DNA from Adenovirus B1, Adenovirus B2, Adenovirus C, Adenovirus 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. DNA samples ($\approx1\times10^3$ copies/reaction) extracted from patient samples were also employed, together with human genomic DNA to validate the performance of the kit to detect DNA targets. **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different pathogens. Due to residual human genomic DNA in the DNA extract samples, several DNA extract samples also show positive results in HEX channel. The primers and probe for the HAdV-B1 assay (**FAM**) also gives a delayed signal (Cq shifted by >5 cycles) in the presence of HAdV-B2 pathogen (see second row, middle and right panels). Thus, if fluorescence signal is observed in both the **FAM** and **TEX615** channels then the proper interpretation is positive for HAdV-B (i.e. HAdV-B1 and/or HAdV-B2), and this is reflected in the Results Interpretation table above.

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.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30_DNA positive control primers. Human genomic DNA doesn't interfere with the detection of the pathogens.

CONTACT US

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

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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] Cy5.5™ is a trademark of GE Healthcare.

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

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