PCRassays.com

ASSAY NAME: PADE_QS
(Pan Human Adenovirus Assay for QuantStudio)

Quantity: 100 x 20µL PCR reactions

2-plex (2 color) assay: detects adenovirus types A, B1, B2, C, D, E, F40, F41 and human RPP30 DNA

SKU #: BUN-PADE-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 (QuantStudio, BioRad). The verification data presented in this PIS were performed with BUN-PADE-D-QS-100 on a QuantStudio™ 7 Pro 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 PADE_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects human adenovirus and human extraction control.

Table of Dyes used in this assay:

Targets	Dyes	Quencher	Refs.
Human adenovirus	FAM	BHQ-1	1, 2
RPP30-DNA control	TAMRA	BHQ-2	3, 4

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

ASSAY HANDLING

The PADE_QS is shipped at ambient temperature, 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: 5X Primer/Probe mix for Human adenovirus and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Mixed positive control: 5000 copies/µl of synthetic 500 bp DNA fragments from human adenovirus D and hRPP30DNA.

Tube 3: InhibiTaq 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). It is important to use an extraction procedure with an appropriate lysis agent.

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

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample or positive control	2
Water (Molecular Biology Grade)	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.

A PCR protocol was used for verification on a QuantStudio™ 7 Pro 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, use the instrument software to determine the quantification cycle, C_q (or use C_T if your instrument does not have the capability to compute a C_q). Fluorescence channels with a C_q < 38 cycles, and final RFU >Threshold is considered "positive" or "+" in the Table below. The "Threshold" value for calling a PCR positive is dependent on the instrument model, well size, and sample volume; thus the user must determine the threshold that is appropriate for their method. For our QuantStudio™ 7 Pro with 96 well plate with 200 µL wells and 20 µL reaction volume, the average RFU was approximately 200,000 and we used a threshold of 20,000 for calling positives or "+" in the Table below.

Human adenovirus FAM™	RPP30 TAMRA™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample doesn't contain human adenovirus DNA. The sample contains human RPP30 DNA.
+	–	The sample contains **human adenovirus** DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains **human adenovirus** DNA and human RPP30 DNA.

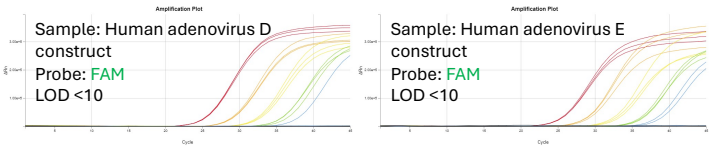
VERIFICATION EXPERIMENTS

The PADE_QS assay verification was carried out as a 2-plex assay, which simultaneously detects human adenovirus DNA and human RPP30 DNA, which serves as a positive extraction-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 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, human genomic DNA, and DNA extracted from patients generously provided by Assurance Scientific Lab (human adenovirus B1, B2, C, and E). **Figure 1** shows the results of these experiments, which indicate that the 2-plex specifically detects the human adenovirus DNA in the human genomic DNA matrix.

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 for human adenovirus (D and E as representatives).

Conclusion: The data in **Figure 1** indicate that the 2-plex primers and probes specifically detect human adenovirus and are also compatible with RPP30_DNA positive control primers. Human genomic DNA doesn't interfere with the detection of pathogens.



Color coding:
10,000 copies
1,000 copies
100 copies
10 copies
1 copy
NTC

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA constructs.

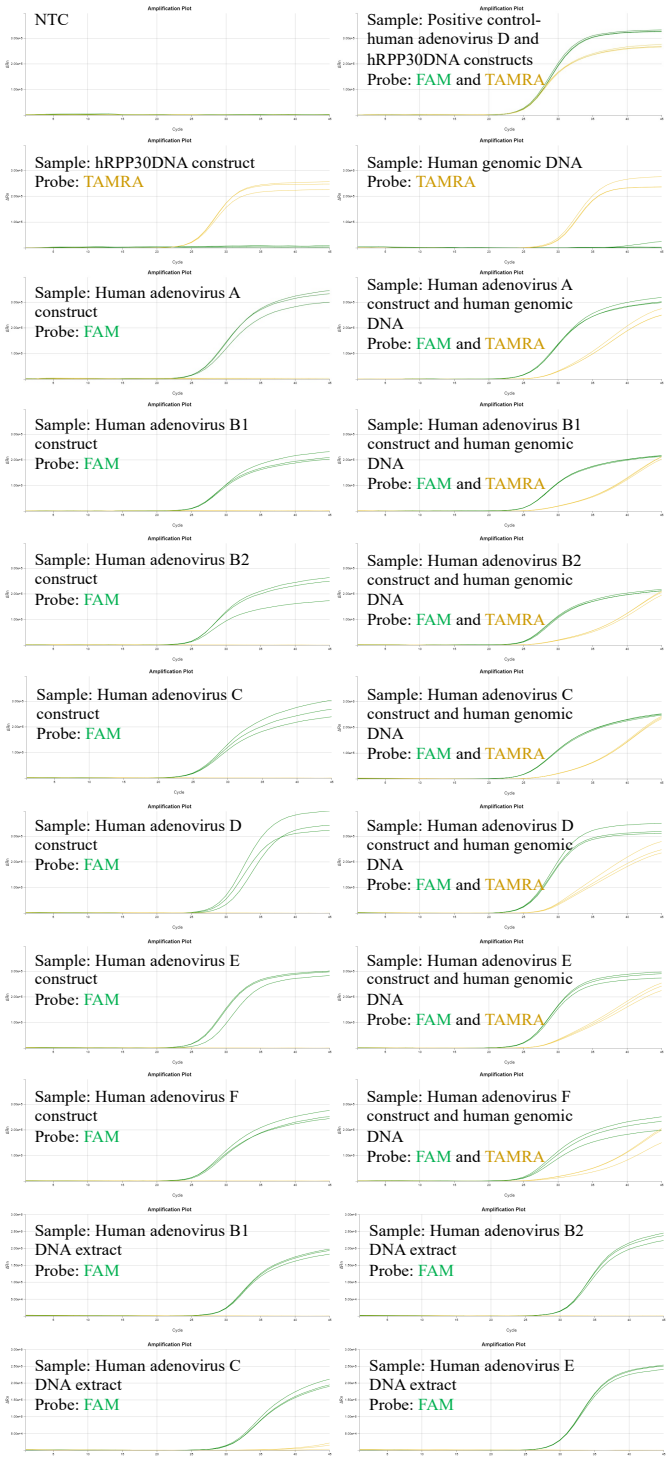


Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

CONTACT US

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

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NOTES

[1] FAM™ (Carboxyfluorescein) is a trademark of Life Techol., Inc.
[2] BHQ-1™ (Black Hole Quencher) trademark of Biosearch Tech., Inc.
[3] TAMRA (Carboxyltetramethylrhodamine) trademark of Applera Cor
[4] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch
[5] "TaqMan" is a trademark of Roche Molecular Systems, Inc.