PCRassays.com

Kit: Adeno Panel2
Quantity: 100 x 20µL PCR reactions

5-plex assay: Adenovirus A, Adenovirus D, Adenovirus F type 40, Adenovirus F type 41 and human RPP30 DNA

Gene: DNA polymerase protein (types A and D), Iva2 protein (F40), and Hexon coat protein (F41)

SKU: PNP-ADE2-D-100

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

CONTENTS

The "Adeno Panel2" kit contains a mixture of primers/probes for detecting Adenovirus A (synonyms: mastadenovirus types 12, 18, 31, 61), Adenovirus D (synonyms: mastadenovirus types 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51, 53, 54, 56, 58, 59, 60, 62, 63, 64, 65, 67, 69, 70, 71, 72, 73, 74, 75), Adendovirus F40 (synonym: mastadenovirus type 40), and Adenovirus F41 (synonym: mastadenovirus type 41). The primers and probes amplify the gene for the DNA polymerase protein (Protein ID: UXO93249.1) in the Adenovirus A genome, the DNA polymerase protein (Protein ID: BBC08480.1) in the Adenovirus D genome, the Iva2 protein (Protein ID: UZE91690.1) in the Adenovirus F type 40 genome, and the hexon coat protein (Protein ID: QJX16372.1) in the Adenovirus F type 41 genome. The primers and probes in Tube 1 are provided as a 5X concentrated working solution. The fluorophore of the Adenovirus F type 40 probe is **FAM™** and the quencher is BHQ-1™.[1,2] The fluorophore of the Adenovirus A probe is **TEX615™** and the quencher is BHQ-2™.[3,4] The fluorophore of the Adenovirus D probe is **Cy5™** and the quencher is BHQ-2™.[5] The fluorophore of the Adenovirus F type 41 probe is **Cy5.5™** and the quencher is BHQ-2™.[6] The same mix also contains primers/probe targeting human RPP30 DNA Intron I (5X concentrated) as a PCR positive control assay for human samples. The fluorophore of the hRPP30DNA probe is **HEX™** and the quencher is BHQ-1™.[7] The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences, Cat number: ITMP-MM-2500).

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of all targets and human RPP30DNA is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

Kit contents:

Tube 1: 5X Primer/Probe mix for Adenovirus A, Adenovirus D, Adenovirus F type 40, Adenovirus F type 41, and hRPP30DNA

Tube 2: 5000 copies/µl Positive controls of synthetic 500 bp DNA of Adenovirus A, Adenovirus D, Adenovirus F type 40, Adenovirus F type 41 and human RPP30DNA.

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

KIT HANDLING AND CONTAMINATION

The Adeno Panel2 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.

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR MasterMix, from Empirical Biosciences. In a reaction with the double positive control, 2 µL of the solution from Tube 2 should be added.

A PCR protocol was used in-house for pre-validation on a Bio-Rad CFX96™ 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 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 < 36 cycles and final RFU >200 is considered "positive" or "+" in the Table below.

Adenovirus F40 FAM™	Adenovirus A TEX615™	Adenovirus D Cy5™	Adenovirus F41 Cy5.5™	RPP30 HEX™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment.
–	–	–	–	+	The sample contains human RPP30 DNA. The sample doesn't contain bacterial DNA.
+	–	–	–	–	The sample contains Adenovirus F40 DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains Adenovirus F40 DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains Adenovirus A DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains Adenovirus A DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains Adenovirus D DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains Adenovirus D DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains Adenovirus F41 DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains Adenovirus F41 DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains Adenovirus F40 DNA, Adenovirus A DNA, Adenovirus D DNA, and Adenovirus F41 DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains Adenovirus F40 DNA, Adenovirus A DNA, Adenovirus D DNA, and Adenovirus F41 DNA. and human RPP30 DNA.

PRE-VALIDATION EXPERIMENTS

The Adeno Panel2 kit validation was carried out as a 5-plex assay, which simultaneously detects DNA from Adenovirus A, Adenovirus D, Adenovirus F type 40, Adenovirus F type 41 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 validation experiments contained 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genomes and the human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 5-plex specifically detects the different Adenovirus serotypes.

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.

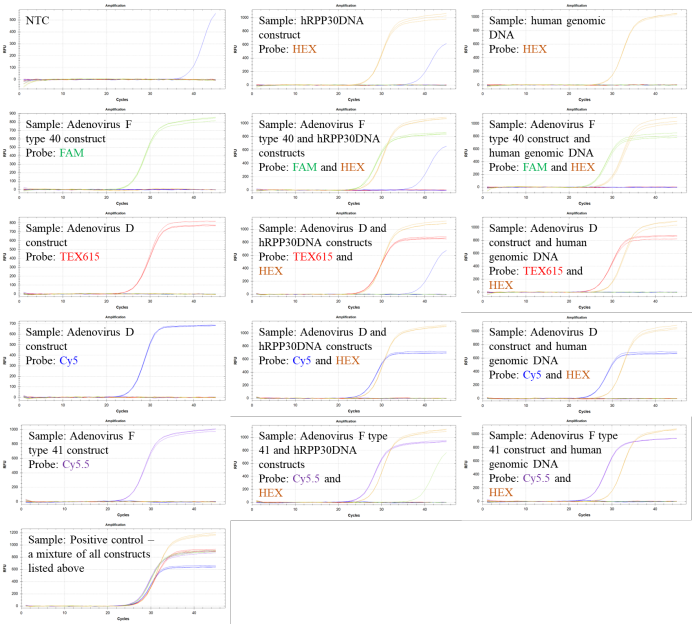

Figure 1: Validation experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **FAM** probe detects Adenovirus F type 40 DNA. The **TEX615** probe detects Adenovirus A DNA. The **Cy5** probe detects Adenovirus D DNA. The **Cy5.5** probe detects Adenovirus F type 41 DNA. The **HEX** probe detects human RPP30 DNA. Amplification from single molecule events was observed in 5 out of 48 wells of the NTC (no template control). The Cq is >36 in these rare NTC positives, so it is considered negative. TapeStation size analysis (not shown) revealed that the artifacts are likely due to contamination from the ubiquitous presence of adenoviruses in the environment.

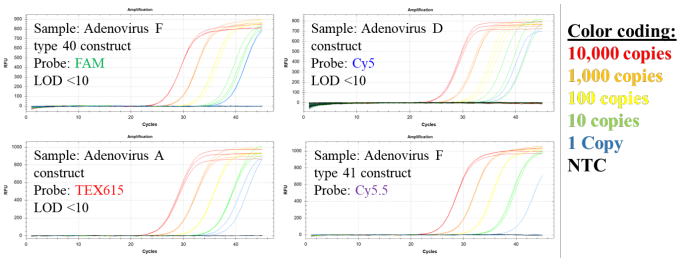

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the bacterial types and are also compatible with DNAS RPP30 DNA positive control primers to detect bacterial targets in human genomic DNA matrix.

CONTACT US

For assistance, please contact DNA Software using the link:
DNA Software Help Center - Jira Service Management

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

Phone: (734) 222-9080

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™ (Sulfo-Cyanine5.5) 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.