Kit: HAdvB1B2CE-RPP30DNA
Quantity: 100 x 20µL PCR reactions

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

Genes: 100 kDa hexon assembly-associated protein (HAdV-B1), DNA polymerase (HAdV-B2), A hypothetical protein (HAdV-C), and Hexon protein (HAdV-E)
SKU#: PNP-ADE1-D-100

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

Kit contents:
Tube 1: 5X Primer/Probe mix for HAdV-B1, HAdV-B2, HAdV-C, HAdV-E, and RPP30 DNA
Tube 2: 5000 copies/µl Positive controls of synthetic DNA for all targets and hRPP30.

CONTENTS

The HAdvB1B2CE-RPP30DNA kit contains a mixture of primers/probes targeting the 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. The primers and probes in Tube 1 are provided as a 5X concentrated working solution. The fluorophore of the HAdV-B1 probe is FAM™ and the quencher is BHQ-1™.[1,2] The fluorophore of the probe for HAdV-B2 is CalFluoRed610™ and the quencher is BHQ-2™.[3,4] The fluorophore of the probe for HAdV-C is Cy5™ and the quencher is BHQ-2™.[5] The fluorophore of the probe for HAdV-E is Cy5.5™ and the quencher is BHQ-2™.[6] The same mix also contains primers/probe targeting human RPP30 DNA (5X concentrated) as a PCR positive control assay for human samples. The fluorophore of the RPP30DNA probe is HEX™ and the quencher is BHQ-1™.[7] The probes are designed as a TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend qPCR Mastermix, from Empirical Bioscience/Fortis Life Sciences, Cat. number: ITMP-MM-2500)

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of all targets and RPP30 DNA and 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**.

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 HAdvB1B2CE-RPP30DNA kit is shipped at ambient temperature, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed. 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 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 qPCR MasterMix, from Empirical Bioscience/Fortis Life Sciences. 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 44xmore

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 is considered "positive" or "+" in the Table below.

HAdV-B1 FAM™	HAdV-B2 CalFluoRed 610™	HAdV-C Cy5™	HAdV-E Cy5.5™	RPP30 HEX™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	–	–	+	The sample does not contain HAdV-B1, HAdV-B2, HAdV-C, or HAdV-E.
+	–	–	–	–	The sample contains HAdV-B1. The sample does not contain HAdV-B2, HAdV-C, or HAdV-E. The sample may not contain spliced human RPP30 DNA.
+	–	–	–	+	The sample contains HAdV-B1 and spliced human RPP30 DNA. The sample does not contain HAdV-B2, HAdV-C, or HAdV-E.
–	+	–	–	–	The sample contains HAdV-B2. The sample doesn't contain HAdV-B1, HAdV-C, or HAdV-E. The sample may not contain spliced human RPP30 DNA.
–	+	–	–	+	The sample contains HAdV-B2 and spliced human RPP30 DNA. The sample does not contain HAdV-B1, HAdV-C, or HAdV-E.
–	–	+	–	–	The sample contains HAdV-C. The sample does not contain HAdV-B1, HAdV-B2, or HAdV-E. The sample may not contain spliced human RPP30 DNA.
–	–	+	–	+	The sample contains HAdV-C and spliced human RPP30 DNA. The sample does not contain HAdV-B1, HAdV-B2, or HAdV-E.
–	–	–	+	–	The sample contains HAdV-E. The sample does not contain HAdV-B1, HAdV-B2, or HAdV-C. The sample may not contain spliced human RPP30 DNA.
–	–	–	+	+	The sample contains HAdV-E and spliced human RPP30 DNA. The sample does not contain HAdV-B1, HAdV-B2, or HAdV-C.
+	+	–	–	–	The sample contains HAdV-B (i.e. HAdV-B1 and/or HAdV-B2). The sample does not contain HAdV-C or HAdV-E. The sample may not contain spliced human RPP30 DNA.
+	+	–	–	+	The sample contains HAdV-B (i.e. HAdV-B1 and/or HAdV-B2). and spliced human RPP30 DNA. The sample does not contain HAdV-C or HAdV-E.
+	–	+	–	–	The sample contains HAdV-B1 and HAdV-C. The sample does not contain HAdV-B2 or HAdV-E. The sample may not contain spliced human RPP30 DNA.
+	–	+	–	+	The sample contains HAdV-B1, HAdV-C and spliced human RPP30 DNA. The sample does not contain HAdV-B2 or HAdV-E.
+	–	–	+	–	The sample contains HAdV-B1 and HAdV-E. The sample does not contain HAdV-B2 or HAdV-C. The sample may not contain spliced human RPP30 DNA.
+	–	–	+	+	The sample contains HAdV-B1, HAdV-E, and spliced human RPP30 DNA. The sample does not contain HAdV-B2 or HAdV-C.
–	+	+	–	–	The sample contains HAdV-B2 and HAdV-C. The sample does not contain HAdV-B1 or HAdV-E. The sample may not contain spliced human RPP30 DNA.
–	+	+	–	+	The sample contains HAdV-B2, HAdV-C, and spliced human RPP30 DNA. The sample does not contain HAdV-B1 or HAdV-E.

HAdV-B1 FAM™	HAdV-B2 CalFluoRed 610™	HAdV-C Cy5™	HAdV-E Cy5.5™	RPP30 HEX™	Recommended Interpretation
–	+	–	+	–	The sample contains HAdV-B2 and HAdV-E. The sample does not contain HAdV-B1 or HAdV-C. The sample may not contain spliced human RPP30 DNA.
–	+	–	+	+	The sample contains HAdV-B2, HAdV-E, and spliced human RPP30 DNA. The sample does not contain HAdV-B1 or HAdV-C.
–	–	+	+	–	The sample contains HAdV-C and HAdV-E. The sample does not contain HAdV-B1 or HAdV-B2. The sample may not contain spliced human RPP30 DNA.
–	–	+	+	+	The sample contains HAdV-C, HAdV-E, and spliced human RPP30 DNA. The sample does not contain HAdV-B1 or HAdV-B2
+	+	+	–	–	The sample contains HAdV-B1, HAdV-B2, and HAdV-C. The sample does not contain HAdV-E. The sample may not contain spliced human RPP30 DNA.
+	+	+	–	+	The sample contains HAdV-B1, HAdV-B2, HAdV-C, and spliced human RPP30 DNA. The sample does not contain HAdV-E.
+	+	–	+	–	The sample contains HAdV-B1, HAdV-B2, and HAdV-E. The sample does not contain HAdV-C. The sample may not contain spliced human RPP30 DNA.
+	+	–	+	+	The sample contains HAdV-B1, HAdV-B2, HAdV-E, and spliced human RPP30 DNA. The sample does not contain HAdV-C.
–	+	+	+	–	The sample contains HAdV-B2, HAdV-C and HAdV-E. The sample does not contain HAdV-B1. The sample may not contain spliced human RPP30 DNA.
–	+	+	+	+	The sample contains HAdV-B2, HAdV-C, HAdV-E and spliced human RPP30 DNA. The sample does not contain HAdV-B1.
+	+	+	+	–	The sample contains HAdV-B1, HAdV-B2, HAdV-C and HAdV-E. The sample may not contain spliced human RPP30 DNA.
+	+	+	+	+	The sample contains HAdV-B1, HAdV-B2, HAdV-C and HAdV-E and spliced human RPP30 DNA.

PRE-VALIDATION EXPERIMENTS

The HAdvB1B2CE-RPP30DNA validation was carried out as a 5-plex assay, which simultaneously detects DNA from HAdV-B1, HAdV-B2, HAdV-C, HAdV-E, and DNA from the human RPP30 DNA gene, 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 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genomes and the spliced human RPP30 DNA gene. 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. The results of these experiments are shown in **Figure 1** below:

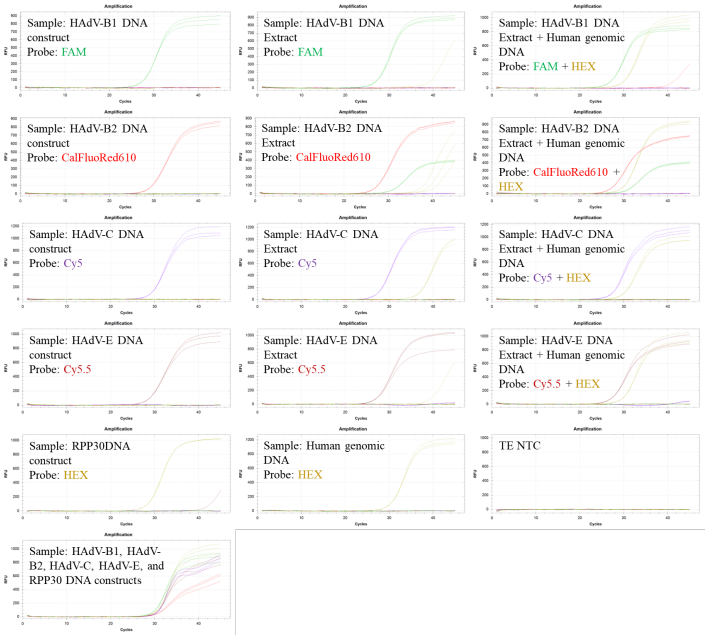

Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but a positive signal is only observed when the target(s) is present in almost all samples, indicating that the amplification is specific. Due to residual human genomic DNA in the DNA extract samples, several DNA extract samples also show positive results in HEX channel. The **FAM** probe detects HAdV-B1. The **CalFluoRed610** probe detects HAdV-B2. 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 **CalFluoRed610** 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 **Cy5** probe detects HAdV-C. The **Cy5.5** probe detects HAdV-E. The **HEX** probe detects spliced human RPP30 DNA.

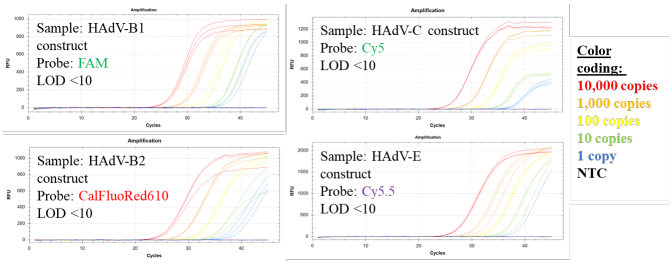

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the HAdV-B1, HAdV-B2, HAdV-C, and HAdV-E primers and probes are compatible with DNAS RPP30 DNA positive control primers and probe in a 5-plex application to detect targets in the matrix of human sample extract.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate. For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction for each target.

CONTACT US

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

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NOTES

[1] FAM™ (Carboxyfluorescein), is a trademark of Life Technologies, Inc

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

3 CalFluorRed610™ (Carboxyfluorescein) is a trademark of Biosearch Technologies, Inc.

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

[5] Cy5.5™, is a trademark of Amersham Biosciences Corp

[6] Cy5™, is a trademark of GE Healthcare.

[7] HEX™ (Hexachloro-fluorescein), is a trademark of Applera Corp.

[8] "TaqMan" is a trademark of Roche Molecular Systems, Inc.