

Human Adenovirus Detection Protocol

A. Introduction

This protocol uses end point PCR[†] with TaqMan™ probes to test for 51 different human adenoviral subtypes. The target sequence is the adenoviral hexon gene (*HEX*), which is highly conserved and specific to human adenoviruses.

PCR run time is 36 min.

B. Equipment

- Spartan DX™ instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

C. Materials

- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- Filtered pipette tips
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- QUANTIPROBES Reaction Mix (Biotools, Cat. No. 10.601)
- Sterile water (DNase-free)
- 1.5 ml microcentrifuge tubes
- Primers which recognize the *HEX* gene (Integrated DNA Technologies)(Table 1)
- TaqMan probe (Biosearch Technologies) for the *HEX* gene (Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>HEX</i> primers [‡]	gCC ACg gTg ggg TTT CTA AAC TT	gCC CCA gTg gTC TTA CAT gCA CAT C	variable
<i>HEX</i> probe [‡]	6FAM-TgC ACC AgA CCC ggg CTC Agg TAC TCC gA -BHQ-1		

6FAM = 6-carboxy-fluorescein, BHQ-1 = Black Hole Quencher 1

‡ Heim A, et al. (2003). Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol.* 70(2):228-239.

Table 1. Primer/probe sequences and amplicon size.

D. Preparation

1. Any commercially-available DNA purification kit may be used to extract adenoviral DNA
2. Turn on Spartan DX instrument and leave it to warm up for a minimum of 10 min
3. Set up thermal cycling program as per Table 2

Step	Temperature	Time	Cycles
Initial denaturation	95.0°C	35 s	1
Denaturation	95.0°C	24 s	50
Annealing/extension	45.0°C	18 s	50

Table 2. Cycling parameters.

E. Protocol

1. Prepare a master mix for *HEX* as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
 - Tube 1: 15 µl of *HEX* master mix
 - Tube 2: 15 µl of *HEX* master mix
 - Tube 3: 15 µl of *HEX* master mix
 - Tube 4: 15 µl of *HEX* master mix
3. In a separate lab area, add the following to each tube:
 - Tube 1: 2.5 µl of purified DNA (sample in question) + 2.5 µl of sterile water
 - Tube 2: 2.5 µl of known adenovirus-positive DNA + 2.5 µl of sterile water (positive control)
 - Tube 3: 2.5 µl of purified DNA (sample in question) + 2.5 µl of known adenovirus-positive DNA
 - Tube 4: 2.5 µl of sterile water (negative control)
4. Mix and spin down reactions
5. Overlay reaction mixture with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert samples into Spartan DX instrument and start your run
8. End point PCR is determined to be positive if the last cycle reading has a fluorescence value greater than 5
9. Example of real-time graph is depicted in Figure 1

Reagent	HEX Master Mix	
	Reaction Formulation	Volume
QUANTIPROBES Reaction Mix (2X)	10 µl x (____+0.5*) Sample #	
Forward primer (10 µM)	1 µl x (____+0.5*) Sample #	
Reverse primer (10 µM)	1 µl x (____+0.5*) Sample #	
Probe (1 µM)	2 µl x (____+0.5*) Sample #	
Sterile water	1 µl x (____+0.5*) Sample #	
Total volume of master mix	15 µl/reaction	____ µl

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mix.

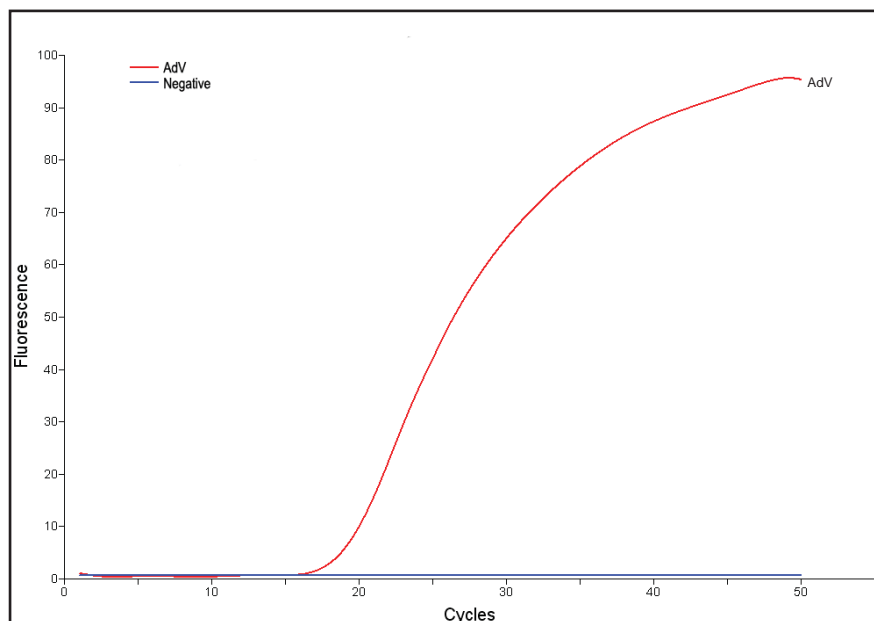


Figure 1. Real-time PCR result for human adenovirus (*HEX* gene).

† - An end point assay is described as an assay that uses data from images collected at the first and last cycles of a PCR run to determine the success or failure of the reaction. End point analysis mode is selected in the options menu of the SpartanDX™.

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