

Enterovirus Detection Protocol

A. Introduction

This assay tests for the presence of Enterovirus using end point PCR[†] with a 6-FAM/BHQ-1 Scorpion® probe. The expected amplicon size is 131 bp. The RNA isolation step is 20 min. PCR run time, including reverse transcription, is 70 min.

B. Equipment

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

C. Materials

- Filtered pipette tips
- 0.2 ml flat-cap PCR tubes (VWR, Cat. No. 53550-106)
- QIAamp Viral RNA Mini Kit (QIAGEN, Cat. No. 52904)
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- AgPath-ID™ One-Step RT-PCR Kit (Ambion, Cat. No. AM1005)
- Sterile water (DNase- and RNase-free)
- Enterovirus-positive RNA sample for positive control
- 1.5 ml microcentrifuge tubes
- Scorpion probe for Enterovirus (Sigma-Genosys)(Table 1)
- Primer for Enterovirus (Integrated DNA Technologies)(Table 1)

D. Preparation

Primer/Probe	Sequences (5'-3')	Amplicon size (bp)
Scorpion probe	(6-FAM)-CgC ggC TCC gTg gTT Agg ATT AgC CgC g-(BHQ-1)- (Spacer-C18)-gAg AAT CCT CCg gCC CCT gAA Tg	131
Reverse primer	ACA AAA ggA AAC ACg gAC ACC CAA Ag	

6-FAM = 6-carboxy-fluorescein, BHQ1 = Black Hole Quencher 1

Table 1. Primer/probe sequences and amplicon size.

- Purify RNA sample with QIAamp Viral RNA Mini Kit as per manufacturer's instructions
- Turn on Spartan DX instrument and let it warm up for a minimum of 10 min
- Set up thermal cycling program as per Table 2

E. Protocol

Step	Temperature	Time	Cycles
Reverse Transcription	45°C	10 min	1
Heat Inactivation	95.5°C	10 min	1
Initial Denaturation	97°C	10 s	1
Denaturation	97°C	33 s	60
Annealing/extension	44°C	18 s	60

Table 2. Reverse transcription and cycling parameters.

1. Prepare Enterovirus master mix, as per Table 3
2. The Spartan DX instrument holds 4 tubes. We recommend setting up the tubes as follows:
 - Tube 1: 13.3 µl of EnV master mix
 - Tube 2: 13.3 µl of EnV master mix
 - Tube 3: 13.3 µl of EnV master mix
 - Tube 4: 13.3 µl of EnV master mix
3. In a separate lab area, add the following
 - Tube 1: 6.7 µl of purified RNA from Sample 1
 - Tube 2: 6.7 µl of purified RNA from Sample 2
 - Tube 3: 6.7 µl of sterile water (negative control)
 - Tube 4: 6.7 µl of Enterovirus-positive RNA sample (positive control)
4. Mix and spin down reaction tubes
5. Overlay reaction mixtures with 15 µl of mineral oil
6. Spin down reaction tubes
7. Insert tubes 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. Representative real-time PCR results are shown in Figure 1

Reagent	EnV Master Mix	
	Reaction Formulation	Volume
RT-PCR Buffer (2X)	10 µl x (____+0.5*) Sample #	
RT-PCR Enzyme Mix (25X)	0.8 µl x (____+0.5*) Sample #	
Detection Enhancer (15X)	1.3 µl x (____+0.5*) Sample #	
Scorpion Probe (10 µM)	0.6 µl x (____+0.5*) Sample #	
Reverse primer (10 µM)	0.6 µl x (____+0.5*) Sample #	
Total volume of master mix	13.3 µl/reaction	____ µl

* Recommended volume correction factor for pipetting error.

Table 3. Components of PCR master mix for EnV.

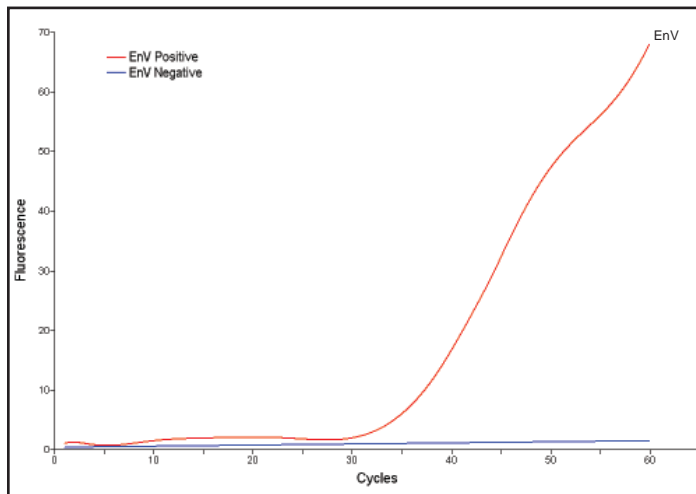


Figure 1. Real-time PCR graph of positive and negative Enterovirus reactions.

† - 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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