

## Protocol: Real-time PCR for Varicella Zoster Virus

### A. Introduction

This protocol uses real-time PCR with TaqMan™ probes to test for Varicella Zoster Virus (VZV). The target sequence in this assay is for the highly conserved *ORF62* region of the VZV genome, which is also specific for VZV.

Approximate real-time PCR run time is 34 min.

### B. Equipment

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

### C. Materials

- 0.2 ml flat-cap PCR tubes (Fisher Scientific, Cat. No. 08-408-214)
- 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 that recognize VZV *ORF62* (Integrated DNA Technologies)(Table 1)
- TaqMan probe (Biosearch Technologies) that recognizes VZV *ORF62* (Table 1)

Primer/Probe	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)
<i>ORF62</i> primers†	CCT Tgg AAA CCA CAT gAT CgT	AgC AgA AgC CTC CTC gAC AA	78
<i>ORF62</i> probe†	6FAM- TCg AAC CCg ggC gTC Cg -BHQ -1		

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

† Cohrs RJ, Gilden DH.(2006).Prevalence and abundance of latently transcribed varicella-zoster virus genes in human ganglia. *J. Virol.* 81(6):2950-6.

**Table 1.** Primer/probe sequences and amplicon size.

### D. Preparation

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

Step	Temperature	Time	Cycles
Initial denaturation	97.0°C	12 s	1
Denaturation	97.0°C	24 s	50
Annealing/extension	45.0°C	16 s	50

**Table 2.** Cycling parameters.

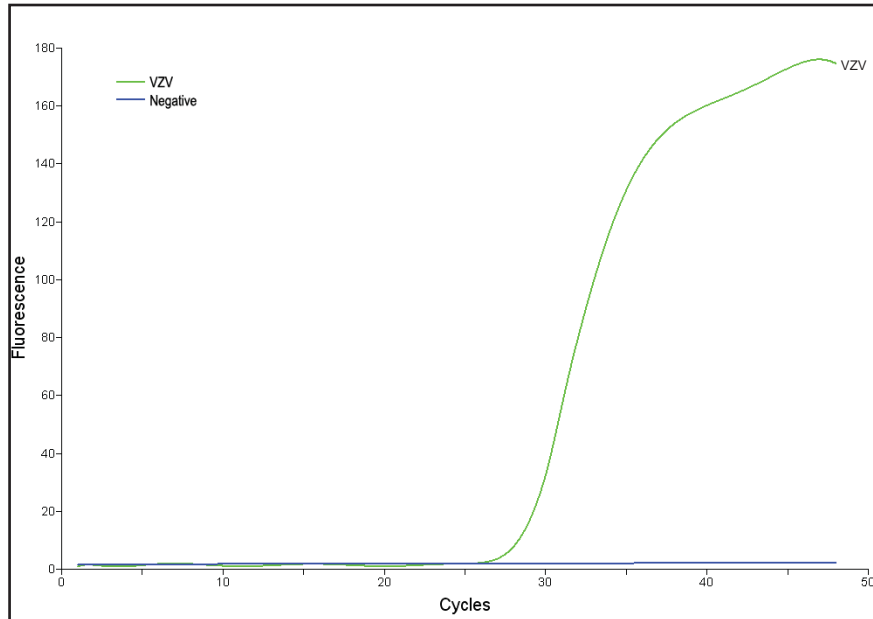
## E. Protocol

1. Prepare a master mix for *ORF62*, as per Table 3
2. To test one unknown sample, we recommend setting up reactions as follows:
  - Tube 1: 17.5 µl of *ORF62* master mix
  - Tube 2: 17.5 µl of *ORF62* master mix
  - Tube 3: 17.5 µl of *ORF62* 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)
  - Tube 2: 2.5 µl of known VZV-positive DNA (positive control)
  - Tube 3: 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. Example of real-time graph is depicted in Figure 1

Reagent	ORF62 Master Mix	
	Reaction Formulation	Volume
QUANTIPROBES Reaction Mix (2X)	10 µl x (____+0.5*) Sample #	
Forward primer (10 µM)	2 µl x (____+0.5*) Sample #	
Reverse primer (10 µM)	2 µl x (____+0.5*) Sample #	
Probe (1 µM)	2 µl x (____+0.5*) Sample #	
Sterile water	1.5 µl x (____+0.5*) Sample #	
<b>Total volume of master mix</b>	<b>17.5 µl/reaction</b>	<b>____ µl</b>

\* Recommended volume correction factor for pipetting error.

**Table 3.** Components of real-time PCR master mix.



**Figure 1.** Real-time PCR result for Varicella Zoster Virus (VZV).

#### Disclaimer

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