



HSV/VZV assay

For the detection of HSV1, HSV2 and VZV using the BD MAX™ system

Instructions for use

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Contents

Introduction	3
Contact information	3
1. Protocol	4
1.1 Materials required	4
1.2 Run settings	4
1.3 Setting up the experiment	5
2. Results interpretation	5



Introduction

This protocol describes the system settings and run setup protocol for the detection of Herpes Simplex virus 1, 2 and Varicella Zoster Virus (HSV1, HSV2 and VZV) on the BD MAX system.

The assay targets the following genes:

Target	Protein
HSV1	Glycoprotein D
HSV2	Glycoprotein G
VZV	Tegument UL21

The HSV/VZV assay was optimized for the BD MAX system by PAMM laboratories, Veldhoven. Validation was performed on swabs (E-swab, Copan) and CSF samples.

Contact information

For information regarding ordering dried snap-in tubes for the HSV/VZV assay:
info@biolegio.com

For information regarding to the protocol:
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1. Protocol

This protocol describes the assay settings that are required for a to run the HSV/VZV assay on the BD MAX system. The HSV/VZV snap-ins contain primers and probes for the detection of HSV1, HSV2 and VZV and require no further preparation. For monitoring failure in the extraction procedure an internal Sample Process Control (SPC) is present in the BD MAX Sample Buffer tube, which is detected using the BD MMK (SPC) mastermix.

1.1 Materials needed

- BD MAX instrument
- BD ExK DNA-1 Extraction kit (BD cat no: 442818)
- BD MMK (SPC) mastermix (BD cat no: 442829)
- BD MAX PCR Cartridges (BD cat no: 437519)
- Dried snap-ins HSV/VZV(Biolegio cat no: BDT-14005)
- Vortex Mixer
- Micropipettes
- Pipette tips with filters
- Disposable gloves
- Lab. coat

1.2 Run settings

The assay is run on the BD MAX with use of the BD MMK(SPC) in combination with the ExK DNA-1 kit for extraction.

Create a full process assay in the test editor named “HSV VZV” using the following channel settings:

The screenshot displays the 'PCR Settings' window in the BD MAX software. The window title is 'Run > Test Editor > Edit > PCR Settings'. The test name is 'HSV/VZV', the extraction type is 'ExK DNA-1 (Plasma/Serum)', and the master mix format is 'Type 1: BD MMK or MMK (SPC) and Dried Primers and Probes'. The interface includes a navigation bar at the top with icons for A and B channels, a status bar showing the date and time (1/4/2016 12:14:24 PM) and user (ADMIN), and a sidebar on the right with buttons for 'Basic Information', 'PCR Settings', 'Melt Settings', 'Test Steps', and 'Result Logic'. The main area contains two tables: 'PCR Settings' and 'Color Compensation'.

PCR Settings Table:

Channel	Wavelength	Alias	PCR Gain	Threshold (EP Min)	Ct Min	Ct Max
PCR	475/520	HSV1	- 40 +	- 100 +	- 0 +	- 0 +
	530/565	HSV2	- 40 +	- 100 +	- 0 +	- 0 +
	585/630	VZV	- 40 +	- 100 +	- 0 +	- 0 +
	630/665		- 0 +	- 0 +	- 0 +	- 0 +
	680/715	SPC	- 40 +	- 100 +	- 0 +	- 0 +

Color Compensation Table:

Excitation Channel	False Receiving Channel				
	Wavelength	475/520	530/565	585/630	630/665
475/520	--	- 0 +	- 0 +	- 0 +	- 0 +
530/565	- 9.4 +	--	- 0 +	- 0 +	- 0 +
585/630	- 0 +	- 0 +	--	- 0 +	- 0 +
630/665	- 0 +	- 0 +	- 0 +	--	- 0 +
680/715	- 0 +	- 0 +	- 0 +	- 0 +	--

At the bottom of the window, there is a warning icon and the text: 'To return melt data, test must have melt gain(s) and at least one melt step.' Below this are 'Save' and 'Cancel' buttons. A 'Back to Test List' button is located in the bottom right corner of the window.



Edit the test steps using the following settings:



1.3 Setting up the experiment

- Create a Work List on the BD MAX instrument using the HSV VZV assay (created in step 1.2) and label the lanes with appropriate sample names.
- Load the prepared Sample Buffer Tubes into their corresponding position in the BD MAX racks.
- Load the BD MAX racks with the corresponding Unitized Reagent Strips. Note: Shake the strip to ensure liquid is at the bottom of tubes.
- Snap-in the BD Extraction tubes (position 1), MMK(SPC) tubes (position 2) and HSV VZV tubes (position 3) into the Reagent Strip.
- Load the racks and cartridges into the BD MAX and Start Run.



2. Results interpretation

- 2.1 For a run to be valid:
- No BD MAX System failures.
 - Negative control (optional) has a Cq value of -1 for all channels except 680/715 (SPC).
 - Positive control (optional) has a Cq values for all channels.
- 2.2 Interpretation if run is valid:
- A Cq value of -1 indicates a negative result.
 - A Cq value of 0 indicates that no Cq value could be obtained. The curve needs to be investigated visually.
 - A Cq value for either of the targets indicates a positive result for the corresponding target.
 - The SPC (channel 680/715) should always give a Cq value. A negative value for the SPC indicates inhibition and therefore this sample should be repeated. However, a positive signal can suppress the SPC amplification. Therefore a positive sample with no amplification for the SPC can be considered to be a valid result.
 - All curves need to be visually checked for right interpretation.

Disclaimer:

PAMM Laboratories is not responsible for the results of the HSV VZV assay on the BD MAX system. Using the “open protocol” (i.e. HSV VZV dried snap in tubes with primer and probes, together with the BD MMK mastermix) the laboratory itself is responsible for the validation of the assay.