ViPrimePLUS Bacterial Vaginosis Multiplex qPCR Kit

Product Code: VMECH002
Pack Size: 100 reactions

INTENDED USE

The ViPrimePLUS Bacterial Vaginosis Multiplex qPCR Kit is a Taqman probe-based multiplex real-time PCR assay for the detection and quantification of Bacterial Vaginosis Associated Bacterium 2 (BVAB- 2) DNA, Atopobium vaginae (A.vagin) DNA and Gardnerella vaginalis (G.vagin) DNA in good quality nucleic acid samples from a variety of sources. This assay is intended for the research use and trained users in molecular biology laboratory environment.

INTRODUCTION

The ViPrimePLUS Bacterial Vaginosis Multiplex qPCR Kit has designed to have very high homology with all reference data within the NCBI database. The kit has the broadest detection profile and detects all clinically relevant strains. The kit detects target genes for Bacterial Vaginosis Associated Bacterium 2, Atopobium vaginae and Gardnerella vaginalis (16S gene, DNA repair protein gene and vaginolysin (vly) gene respectively.

PRINCIPLE OF TEST

The kit contains the target specific primers and Taqman® probe, and this can be detected through the FAM, ROX, VIC/HEX channels.

The kit is mainly used for human-related samples. The Endogenous Control (EC) is the gene within an organism; the kit provides the Endogenous Control primers and probe to be run in the same reaction and the EC amplicons are detected via Cy5 channel.

A positive control with different genomes is provided in one single tube. A positive result indicates that the primers and probes for detecting the target gene worked properly in the particular experiment. It can also be used as qualitative analysis control of the experimental set-up. The positive control does not have the Endogenous Control sequence. The positive control does not expect positive result at EC channel. Extra care must be taken to avoid cross-contamination.

QUALITY CONTROL

Each lot of ViPrimePLUS Bacterial Vaginosis Multiplex qPCR Kit has been tested against predetermined specifications to ensure consistent product quality under ISO 9001:2015 – certified Quality Management System.

SENSITIVITY & SPECIFICITY

The detection limit is tested to have at least 100 copies per reaction. The primers and probe are 100% specific.

STORAGE & STABILITY

Store at -20ºC upon arrival and avoid light exposure. Stable at -20°C up to the expiry date stated. Keep in aliquot to reduce freeze-thaw cycles. Always store the kit at -20°C once the lyophilized tube has reconstituted. Ship and store at ambient temperature not more than 10 days.

LIMITATION OF TEST

For research use only. Not recommended for diagnosis of disease in humans or animals.
Result is dependent on the yield and quality of the nucleic acids extracted from the method of extraction. Thus, it is important to do spectrophotometric and gel analysis on the extracted samples.

KIT COMPONENTS

Kit Components	Tube Colour	Volume (ml)
Primers and Probe Mix (gene PPM) BVAB- 2 specific PPM – FAM probe A.vagin specific PPM – HEX probe G.vagin specific PPM – ROX probe Endogenous Control PPM – Cy5 probe	Amber	0.1 ml
Multi-Positive Control Template	Pink	Lyophilized
2X PCR Master Mix	Purple	Lyophilized
Nuclease-free Water	White	1.5 ml
Template Preparation Buffer	Orange	1.5 ml
Master Mix Preparation Buffer	Transparent	1.5 ml

PROTOCOL 1

Reconstitution of Reagents

*Pulse-spin each tube prior to opening.

Components	Volume	Reagents
Positive Control Template	500 µl	Template Preparation Buffer
2X PCR Master Mix	1.1 ml	Master Mix Preparation Buffer

PROTOCOL 2

DNA extraction

The kit will work well with high quality DNA extracted from any extraction kit with minimal PCR inhibitors present.

Real-time PCR reaction set-up

Recommended real-time PCR reaction set-up:

Reagents	1 reaction (µl)
2X PCR Master Mix	10
Gene Primers and Probe Mix	1
Sample DNA	5 – 9
Nuclease-free water	Top up to 20 µl

**Suggested sample concentration 5-100ng/µl.*

**Adjust Nuclease-free Water to make up the final reaction volume of 20 µl.*

**Prepare an extra reaction to accommodate for pipetting error.*

If ROX passive reference dye is needed for the real-time PCR system, the passive reference detection is suggested to be **turned off or set to **"none"** indicating no passive reference.

Negative control

Set aside one tube/well as negative control where 9 µl of Nuclease-free Water is used as the template. This is also known as no template control.

Positive control

For qualitative analysis, set aside one tube as positive control where 9 µl of the positive control is used as the template

PROGRAM

Set the thermal cycler parameters as follows:

Step	Time	Temp	Cycles	Scan
Enzyme activation	3 mins	95°C		
Denaturation	15 secs	95°C	45	
Anneal/Elongation	1 min	60°C		√*

*Make sure to collect fluorogenic data through FAM, HEX, ROX, and Cy5 channels during this step

INTERPRETATION OF RESULTS

Pathogen specific amplification signal are detected via FAM, VIC/HEX, and ROX channel, while EC amplification is detected via Cy5 channel. The signal is positive if the amplification curve crosses the threshold line. The result is relevant provided both positive and negative controls give valid results.

When analysing sample Ct values, the threshold is checked within the run file before interpreting the data. The suggested threshold is set to 10% of the relevant positive control End Point Fluorescence.

Summary of interpretation:

Target	EC (Cy5 channel)	Negative Control	Positive Control	Interpretation
+; ≤35	+; ≤31	-	+; ≤21	Valid, positive
+; ≤35	-	-	+; ≤21	Valid, positive
-	+; ≤31	-	+; ≤21	Valid, negative
-	-	-	-	Invalid
+; ≤35	+; ≤31	+	+; ≤21	Invalid

EC	BVAB- 2 Sample (FAM channel)	A.vagin Sample (HEX channel)	G.vagin Sample (ROX channel)	Interpretation
+ / -	+; ≤31	-	-	Valid, positive for BVAB- 2
+ / -	-	+; ≤31	-	Valid, positive for A.vagin
+ / -	-	-	+; ≤31	Valid, positive for G.vagin
+	-	-	-	Negative result
-	-	-	-	Invalid test

Positive signals observed in multiple channels represents that a sample contains more than one target pathogen.

Positive Control

When used accordingly and assuming between 90% to 110% efficiency, the undiluted positive control should amplify in a Ct value of 18±3. If running a positive control standard curve for a quantitative result, an efficiency between 90% to 110% will provide most accurate quantification. If both fall outside of the range, the repeated test is suggested to be repeated. The positive control does not include endogenous control sequence; therefore the positive control should not amplify in the internal control channel.

Endogenous Control

Endogenous Control results are useful to interpret the validity of test for the samples which deliver negative result. When used accordingly and assuming 100% extraction efficiency, a Ct value of 28±3 is within normal range with the indication of a good extraction. The Ct value from EC can be vary according to the DNA yield and purity in the sample. A high genome copy amplification may out compete the EC amplification. Thus, the latter may not produce an amplification signal. The positive result is still valid in this case.

Negative Control

The negative control should not have Ct value and achieve flat line. However, it is not uncommon for laboratory contamination to have very late signal. If the negative control is having ≥5 Ct values later than the sample signal, then it can be considered negative and the sample result can be considered as valid. If the negative control is ≤5 Ct values, the result is considered as invalid.

Positive Samples

The cut off Ct value of the samples is ≤35; suggested to have defined “sigmoidal” amplification plot delivered. In order to present the results in target copy number, a quantitative result and signal can be calculated by comparing the signal to the positive control standard curve.

WARRANTY AND LIMITED LIABILITY

The performance characteristics stated were obtained using the assay procedure in this insert. Failure to comply with the instructions may derive inaccurate results. In such event, manufacturer disclaims all warranty expressed, implied or statutory including the implied warranty of merchantability and the fitness of use.

The manufacturer will not be liable for any damage caused by misuse, improper handling and storage; non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.