PCRassays.com

ASSAY NAME: AVAG-D-BR

Quantity: 100 x 20µL PCR reactions

2-plex assay: *Atopobium vaginae* (also known as *Fannyhessea vaginae*) and human RPP30 DNA

SKU#'s: PNP-AVAG-D-BR-100 (Bio-Rad)

(RUO). Research Use Only. Not for use in Diagnostic Procedures.

SCOPE OF THIS PRODUCT INFORMATION SHEET (PIS)

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio, MIC). The verification data presented in this document were performed using PNP-AVAG-D-BR-100 on a BioRad CFX96. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you are planning to use a different qPCR instrument.

CONTENTS

The primers and probes in the AVAG assay are provided in Tube 1 as a 20X concentrated working solution that detects *A. vaginae* and a human extraction control.

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
A. vaginae	**FAM**	BHQ-1	1, 2
RPP30-DNA control	**HEX**	BHQ-1	2, 3

The probes are designed as a TaqMan[4] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING AND CONTAMINATION

The AVAG bundle is shipped on ice, and should be stored at -20 °C. The bundle should be kept on ice once thawed. Do not subject the enzyme to multiple freeze-thaw cycles.

Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

Assay contents:

Tube 1: Primer/Probe mix (20X) for *A. vaginae* and hRPP30DNA.

Tube 2: Positive control: Synthetic 500 bp DNA fragments of *A. vaginae* and human RPP30DNA.

Tube 3: InhibiTaq Standard qPCR enzyme Mastermix (enough for 100 rxns. with 20 µL total volume). This is a custom formulation from Fortis Life Sciences to the specifications of PCRassays.com.

EXPERIMENTAL

Set up your reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq Standard qPCR enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water (Molecular Biology Grade)	7

Notes: To improve assay sensitivity, up to 9 µL of sample can be added (water volume adjusted accordingly) for a total reaction volume of 20 µL. For positive control rxns., add 2 µL of the solution from Tube 2.

A PCR protocol was used for verification on a Bio-Rad CFX96™ Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 95 °C for 2 minutes
2	Incubate @ 95 °C for 3 seconds
3	Incubate @ 55 °C for 15 seconds
4	Plate Read
5	Go to Step 2, repeat 44xmore

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 38 cycles and final RFU > "threshold" is considered "positive" or "+" in the Table below. The "threshold" is 200 on BioRad instruments.

A. vaginae **FAM™**	hRPP30 **HEX™**	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample does not contain bacterial DNA of interest. The sample contains human RPP30 DNA.
+	–	The sample contains ***A. vaginae*** DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains ***A. vaginae*** DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The AVAG primers and probes were previously verified as part of the study of a 5-plex assay (SKU#: PNP-WH3-D-BR-100), which simultaneously detects DNA from *Atopobium vaginae*, *Gardnerella vaginalis*, Megasphaera type 1, Megasphaera type 2, and human RPP30 DNA, which serves as a positive extraction-control assay. Data from the *A. vaginae* and control channel are presented below to show the performance expected for the AVAG kit.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples for verification experiments contained 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genome and the human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1**.

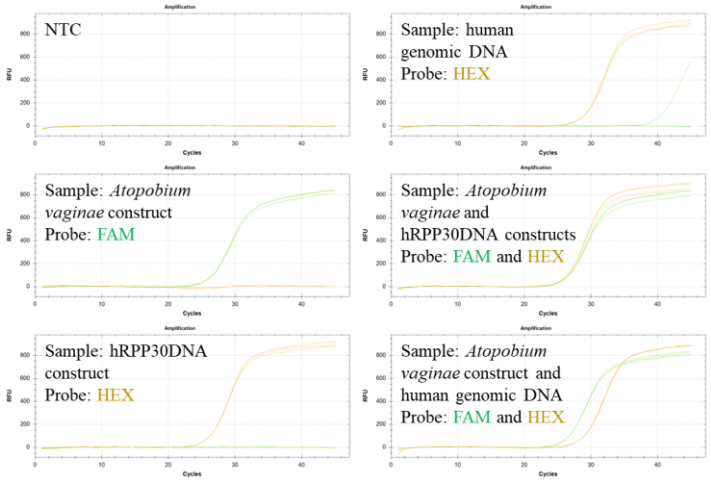

Figure 1: Verification experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **FAM** probe detects *A. vaginae*. The **HEX** probe detects human RPP30 DNA.

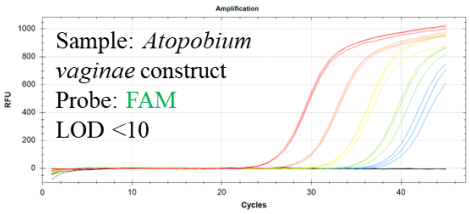

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicates that the *A. vaginae* primers and probe are compatible with DNAS RPP30 DNA positive control primers and probe in the human genomic DNA matrix.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate. For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction for each target.

CONTACT US

For assistance, please contact DNA Software using the link: https://dnasoft.jira.com/servicedesk/customer/portals

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NOTES

[1] FAM™ (Carboxyfluorescein) is a trademark of Life Technologies, Inc.

[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

[3] HEX™ (Hexachloro-fluorescein) is a trademark of Applera Corp.

[4] "TaqMan" is a trademark of Roche Molecular Systems, Inc.