Kit: T.vag-RPP30DNA
Quantity: 100 x 20µL PCR reactions

2-plex assay: *Trichomonas vaginalis* and human RPP30 DNA

Gene: Protein phosphatase 2C family
SKU: PNP-TVA-D-100

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

Kit contents:

Tube 1: 20X Primer/Probe mix for *Trichomonas vaginalis* and hRPP30DNA.

Tube 2: 5000 copies/µl Positive controls of synthetic 500 bp DNA for *Trichomonas vaginalis* and human RPP30DNA.

CONTENTS

The T.vag-RPP30DNA kit contains a mixture of primers/probe targeting the gene of the protein phosphatase 2C family (Protein ID: XP_001315231.1) in the genome of the protozoan parasite *Trichomonas vaginalis*. The primers and probes in Tube 1 are provided as a 20X concentrated working solution. The fluorophore of the *T. vaginalis* probe is **Cy5™** and the quencher is BHQ-2™.[1,2] The same mix also contains primers/probe targeting human RPP30DNA Intron I (20X concentrated) as a PCR positive control assay for human samples. The fluorophore of the RPP30DNA probe is **HEX™** and the quencher is BHQ-1™.[3,4] The probes are designed as TaqMan[5] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences, Cat number: ITMP-MM-2500).

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of *T. vaginalis* and human RPP30DNA is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.

KIT HANDLING AND CONTAMINATION

The T.vag-RPP30DNA kit is shipped at ambient temperature, and should be stored at approximately -20°C. The kit 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.

EXPERIMENTAL

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

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

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR MasterMix, from Empirical Biosciences. In a reaction with the double positive control, 2 µL of the solution from Tube 2 should be added.

A PCR protocol was used in-house for pre-validation 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 44× more

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 >200 is considered "positive" or "+" in the Table below.

Trichomonas vaginalis Cy5™	RPP30 HEX™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample contains human RPP30 DNA. The sample doesn't contain *Trichomonas vaginalis* DNA.
+	–	The sample contains *Trichomonas vaginalis* DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains *Trichomonas vaginalis* DNA and human RPP30 DNA.

PRE-VALIDATION EXPERIMENTS

The T.vag-RPP30DNA kit validation was carried out as a 2-plex assay, which simultaneously detects DNA from *T. vaginalis* and human RPP30 DNA, which serves as a positive extraction-control assay.

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

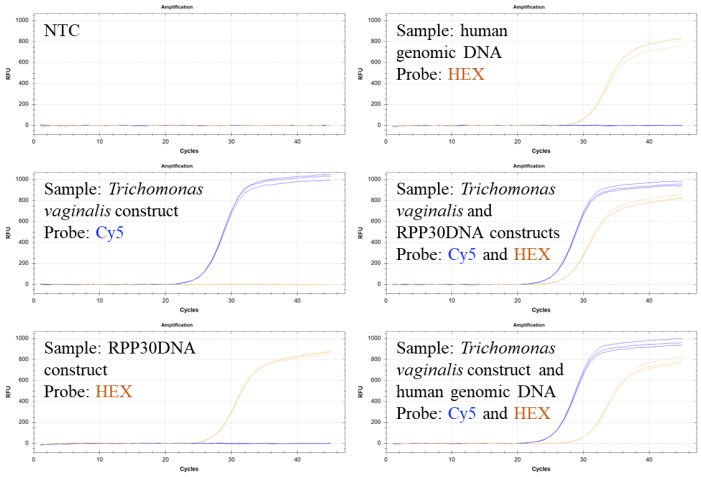

Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but a positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **Cy5** probe detects *T. vaginalis* DNA. The **HEX** probe detects human RPP30 DNA.

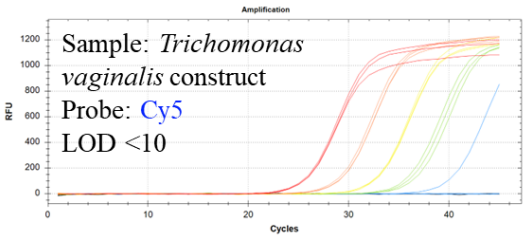

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the *T. vaginalis* primers and probe are compatible with DNAS RPP30 DNA positive control primers and probe in a 2-plex application to detect *T. vaginalis* in the matrix of human sample extract.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080

NOTES

[1] Cy5™ is a trademark of GE Healthcare.

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

[3] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.

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

[5] TaqMan™ is a trademark of Roche Diagnostics, Inc.