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ASSAY NAME: STI11_QS (Genital Ulcer 2) Panel

Quantity: 100 x 20µL PCR reactions
5-plex assay: HSV1, HSV2, *Treponema pallidum*, *Haemophilus ducreyi*, and human RPP30 DNA

SKU #: PNP-GUPN-D-QS-100 (QuantStudio)

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

SCOPE OF THIS DOCUMENT:

The oligonucleotide recipes are optimized for QuantStudio. The verification data presented in this product information sheet were performed using PNP-GUPN-D-QS-100 on a QuantStudio 7 Flex Real-Time System. The performance of the other QuantStudio instruments should be similar (presuming they are equipped with the proper wavelength filters). Contact PCRassays.com if you are planning to use a different instrument.

CONTENTS

The primers and probes in the STI11 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
HSV1	**FAM**	BHQ-1	1,2
H. ducreyi	**HEX**	BHQ-1	3
RPP30-DNA control	**TAMRA**	BHQ-1	4
T. pallidum	**TEX615**	BHQ-2	5, 6
HSV2	**Cy5**	BHQ-2	7

The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

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

ASSAY HANDLING AND CONTAMINATION

The STI11 assay is shipped at ambient temperature, and should be stored at -20 °C. The assay 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: 5X Primer/Probe mix for HSV1, HSV2, *Treponema pallidum, Haemophilus ducreyi,* and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for HSV1, HSV2, *T. pallidum, H. ducreyi*, and hRPP30DNA.

Tube 3: Spike-in control. 1.0E6 copies/uL of synthetic 500 BP human RPP30 gene. **Do not add directly to the PCR reaction!**

Tube 4: InhibiTaq 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

(Optional) add 1 µL of spike-in control (Tube 3) to each specimen before extraction. **Do not add directly to the PCR reaction!** It serves as extraction and PCR reaction control.

Perform nucleic acid extraction/purification (recommended). Set up your PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

Notes: To improve assay sensitivity, up to 6 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 (i.e., the "sample").

A PCR protocol was used for verification on a QuantStudio™ 7 Flex Real-Time System, with the following program:

Step	Thermocycling Protocol:
1	Incubate @ 85 °C for 1 second
2	Incubate @ 94 °C for 2 minutes
3	Incubate @ 94 °C for 3 seconds
4	Incubate @ 55 °C for 22 seconds
5	Plate Read
6	Incubate @ 85 °C for 1 second
7	Incubate @ 94 °C for 3 seconds
8	Incubate @ 55 °C for 22 seconds
9	Plate Read
10	Go to Step 6, repeat 43× 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 >200,000 is considered “positive” or “+” in the Table below.

HSV1 FAM™	*H. ducreyi* HEX™	*T. pallidum* TEX615™	*HSV2* Cy5™	hRPP30 TAMRA™	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 *HSV1* DNA. The sample may not contain human RPP30 DNA.
+	−	−	−	+	The sample contains *HSV1* DNA and human RPP30 DNA.
−	+	−	−	−	The sample contains ***H. ducreyi*** DNA. The sample may not contain human RPP30 DNA.
−	+	−	−	+	The sample contains ***H. ducreyi*** DNA and human RPP30 DNA.
−	−	+	−	−	The sample contains *T. pallidum* DNA. The sample may not contain human RPP30 DNA.
−	−	+	−	+	The sample contains *T. pallidum* DNA and *human* RPP30 DNA.
−	−	−	+	−	The sample contains *HSV2* DNA. The sample may not contain human RPP30 DNA.
−	−	−	+	+	The sample contains *HSV2* DNA and human RPP30 DNA.
+	+	+	+	−	The sample contains *HSV1*, ***H. ducreyi***, *T. pallidum*, *HSV2* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *HSV1*, ***H. ducreyi***, *T. pallidum*, *HSV2* DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The STI11 assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from HSV1, *T. pallidum, H. ducreyi,* HSV2, 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 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 pathogen genomes, human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 5-plex specifically detects the different bacterial species in the human genomic DNA matrix.

NOTES

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

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

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

[4] TAMRA (Carboxyltetramethylrhodamine), a trademark of Applera Corp.

[5] TEX615™ is a trademark of Thermo Fisher Scientific.

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

[7] Cy5™, a trademark of GE Healthcare.

[8] “TaqMan” is a trademark of Roche Molecular Systems, Inc.

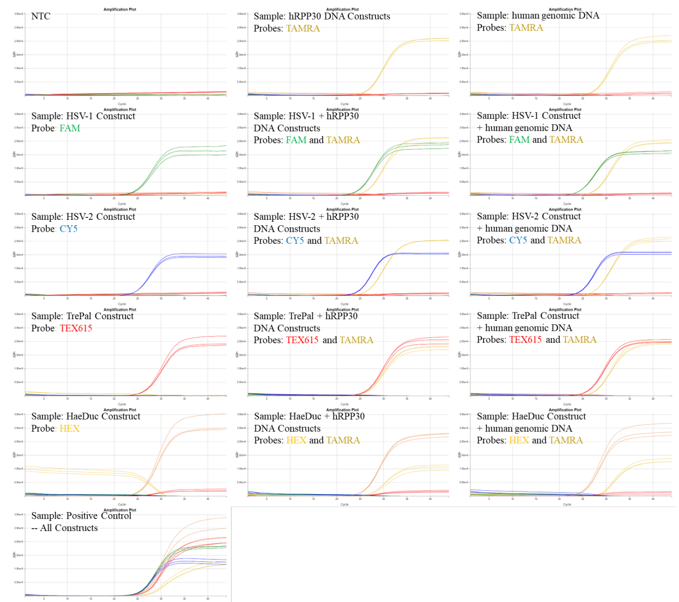

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

The 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) <100 copies/reaction.

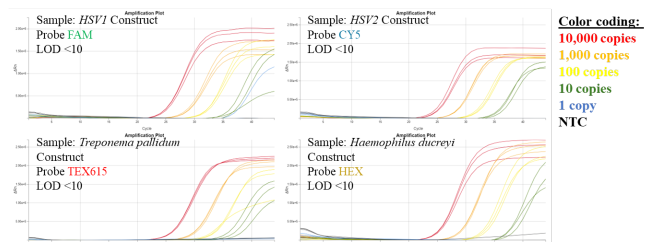

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the bacterial types and are also compatible with RPP30_DNA positive control primers. Human genomic DNA matrix doesn’t affect detection of pathogen DNA.

CONTACT US

For assistance, please contact DNA Software using the link: https://www.pcrassays.com/contact/

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