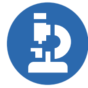
SZABO SCANDIC
Part of Europa Biosite

Produktinformation

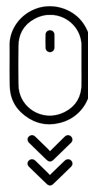
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien
T. +43(0)1 489 3961-0
F. +43(0)1 489 3961-7
mail@szabo-scandic.com
www.szabo-scandic.com

PCRassays.com

ASSAY NAME: STI8

Quantity: 100 x 20µL PCR reactions

5-plex assay: *Treponema pallidum, Haemophilus ducreyi*, *Chlamydia trachomatis*, Herpes simplex virus type 2, and human RPP30 DNA

SKU: PNP-STI8-D-BR-100 (Bio-Rad)
PNP-STI8-D-QS-100 (QuantStudio)

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

SCOPE OF THIS IFU:

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio). The pre-validation data presented in this IFU were performed using PNP-STI8-D-QS-100 on a Bio-Rad CFX96. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you need to use a different qPCR instrument.

CONTENTS

The primers and probes in the STI8 assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human extraction control.

Table of Dyes used in this assay:

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

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

ASSAY HANDLING AND CONTAMINATION

The STI8 assay is shipped at ambient temperature, and should be stored at -20 °C. The tubes 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.

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

Assay contents:

__Tube 1__: Primer/Probe mix (5X) for *T. pallidum*, *H. ducreyi*, *C. trachomatis*, HSV-2, and hRPP30DNA.

__Tube 2__: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of *T. pallidum*, *H. ducreyi*, *C. trachomatis*, HSV-2, and hRPP30DNA.

__Tube 3__: Spike-in control. 1.0E6 copies/uL of synthetic 500 BP human RPP30 gene.

__Tube 4__: 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

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

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

Component	Volume (µL)
InhibiTaq qPCR 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 BioRad CFX96 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

For QuantStudio instruments, we recommend a Step 3 cycle time of 22 seconds at 55 °C.

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 and 200,000 on QuantStudio 5, 6, 7, 12K instruments.

T. pallidum FAM™	*H. ducreyi* TEX615™	*C. trachomatis* Cy5™	HSV-2 Cy5.5 ™	RPP30 HEX™	Recommended Interpretation
−	−	−		−	The PCR reaction failed. Please repeat the experiment.
−	−	−		+	The sample contains human RPP30 DNA. The sample doesn't contain bacterial 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 *H. ducreyi* DNA. The sample may not contain human RPP30 DNA.
−	+	−		+	The sample contains *H. ducreyi* DNA and human RPP30 DNA.
−	−	+		−	The sample contains *C. trachomatis* DNA. The sample may not contain human RPP30 DNA.
−	−	+		+	The sample contains *C. trachomatis* DNA and human RPP30 DNA.
-	-	-	+	-	The sample contains HSV-2 DNA. The sample may not contain human RPP30 DNA.
-	-	-	+	+	The sample contains HSV-2 DNA and human RPP30 DNA.
+	+	+	+	−	The sample contains *T. pallidum* DNA, *H. ducreyi* DNA, *C. trachomatis* DNA, and HSV-2 DNA.The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *T. pallidum* DNA, *H. ducreyi* DNA, *C. trachomatis* DNA, HSV-2 DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The STI8 verification was carried out as a 5-plex assay, which simultaneously detects DNA from *Treponema pallidum*, *Haemophilus ducreyi*, *Chlamydia trachomatis*, Herpes simplex virus type 2, and human RPP30 DNA, which serves as a positive 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 DNA constructs (from Twist Biosciences) harboring the regions of interest from the pathogen genomes, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 5-plex specifically detects the different pathogens.

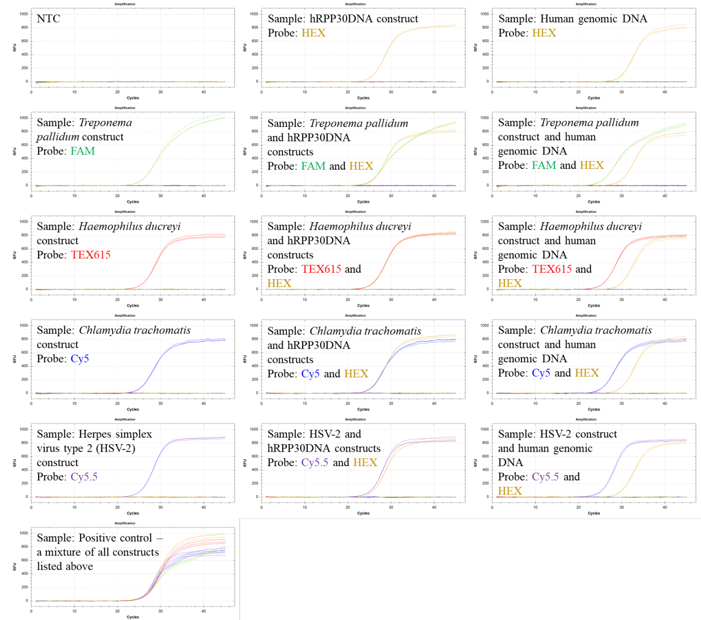


Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

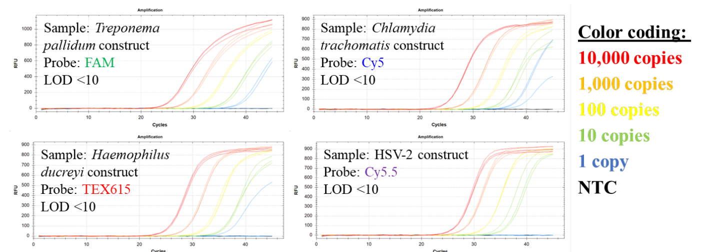


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the pathogens and are also compatible with RPP30_DNA positive control primers. Human genomic DNA doesn't interfere with the detection of the pathogens.

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.

NOTES

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

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

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

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

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

[6] Cy5.5™ is a trademark of GE Healthcare.

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

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

CONTACT US

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

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

Phone: (734) 222-9080