PCRassays.com

ASSAY NAME: Wound8

Quantity: 100 x 20µL PCR reactions

5-plex assay: *Proteus vulgaris, Morganella morganii, Serratia marcescens, Staphylococcus saprophyticus*, and human RPP30 DNA

SKU: PNP-WND8-D-BR-100 (Bio-Rad)
PNP-WND8-D-QS-100 (QuantStudio)

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

SCOPE OF THIS PRODUCT INFORMATION SHEET:

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio). The verification data presented in this IFU were performed using PNP-Wound8-D-BR-100 on a Bio-Rad CFX 96. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you are planning to use a different instrument.

CONTENTS

The primers and probes in the Wound8 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.
P. vulgaris	FAM	BHQ-1	1,2
RPP30-DNA control	HEX	BHQ-1	7
M. morganii	TEX615	BHQ-2	3,4
S. marcescens	Cy5	BHQ-2	5
S. saprophyticus	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 Wound8 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, which is NOT included in this assay.

ASSAY CONTENTS

Tube 1: 5X Primer/Probe mix for *P. vulgaris*, *M. morganii, S. marcescens*, *S. saprophyticus*, and hRPP30DNA

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments of *P. vulgaris, M. morganii, S. marcescens*, *S. saprophyticus*, and human RPP30DNA.

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 the 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 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 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

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

P. vulgaris FAM™	*M. morganii* TEX615™	*S. marcescens* Cy5™	*S. saprophyticus* 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 *P. vulgaris* DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains *P. vulgaris* DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *M. morganii* DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains *M. morganii* DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains *S. marcescens* DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains *S. marcescens* DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains *S. saprophyticus* DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains *S. saprophyticus* DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains *P. vulgaris* DNA, *M. morganii DNA*, *S. marcescens DNA*, and *S. saprophyticus* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *P. vulgaris* DNA, *M. morganii DNA*, *S. marcescens DNA*, and *S. saprophyticus* DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The Wound8 assay verification was carried out as a 4-plex assay, which simultaneously detects DNA from *P. vulgaris, M. morganii, S. marcescens*, *S. saprophyticus*, 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.

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.

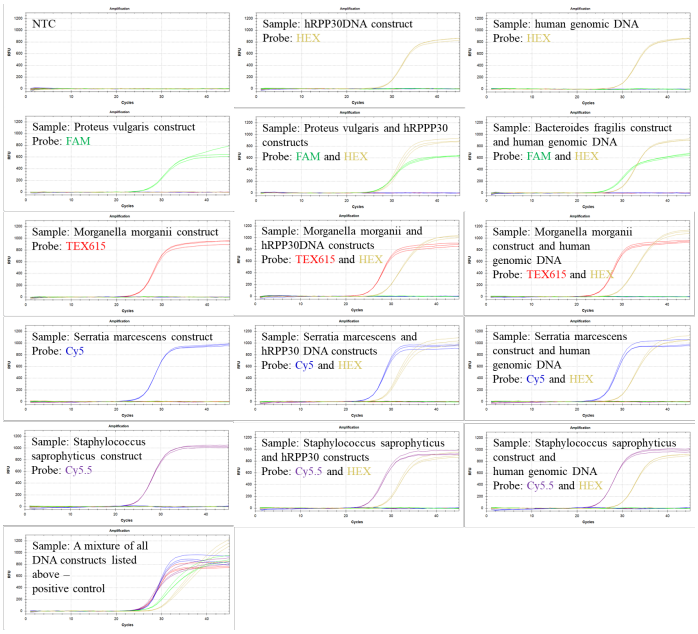

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

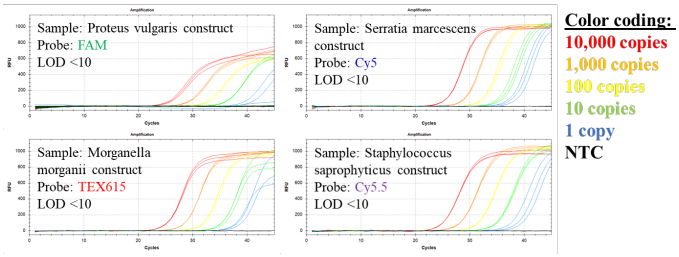

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

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.

CONTACT US

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

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NOTES

[1] FAM™ (Carboxyfluorescein), a trademark of Life Technologies Corp.
[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™ (Sulfo-Cyanine5.5) 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.