

**ASSAY NAME: WH4 (Women's Health 4)**

**Quantity: 100 x 20µL PCR reactions**

**5-plex assay: *Enterococcus faecium*, *Mobiluncus curtisii*, *Prevotella bivia*, *Streptococcus agalactiae*, and human RPP30 DNA**

**SKU: PNP-WH4-D-BR-100 (Bio-Rad)  
PNP-WH4-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-WH4-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 qPCR instrument.

### CONTENTS

The primers and probes in the Women's Health 4 (WH4) assay are provided in Tube 1 as a 5X concentrated working solution that detect 4 pathogens and a human control.

#### Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
<i>S. agalctiae</i>	<b>FAM</b>	BHQ-1	1,2
RPP30-DNA control	<b>HEX</b>	BHQ-1	7
<i>E. faecium</i>	<b>TEX615</b>	BHQ-2	3,4
<i>M. curtisii</i>	<b>Cy5</b>	BHQ-2	5
<i>P. bivia</i>	<b>Cy5.5</b>	BHQ-2	6

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

### ASSAY HANDLING AND CONTAMINATION

The Women's Health 4 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 *E. faecium*, *M. curtisii*, *P. bivia*, *S. agalactiae*, and hRPP30DNA.

**Tube 2:** 5000 copies/µl Positive controls of synthetic 500 bp DNA for *E. faecium*, *M. curtisii*, *P. bivia*, *S. agalactiae*, 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 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 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 44x more

For QuantStudio instruments, we recommend a 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, C<sub>q</sub>. (C<sub>q</sub> is preferred over Ct). Each fluorescence channel with a C<sub>q</sub> < 38 cycles and final RFU > 200 (for BioRad CFX instruments) is considered “positive” or “+” in the Table below. Note: On QuantStudio 5, 6, 7, 12K instruments the final RFU cutoff is >200,000.

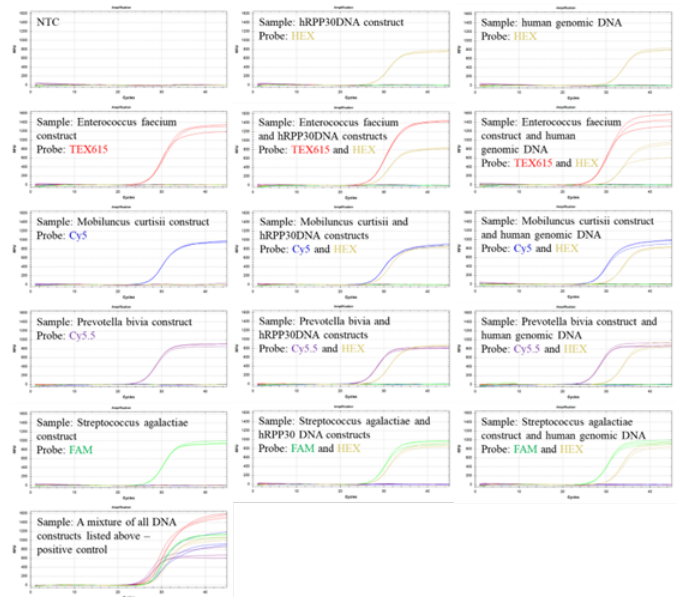
<i>B. bronchiseptica</i> TEX615™	<i>M. curtisii</i> Cy5™	<i>P. bivia</i> Cy5.5™	<i>S. agalactiae</i> FAM™	hRPP30 HEX™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	–	–	+	The sample does not contain bacterial DNA of interest. The sample contains human RPP30 DNA.
+	–	–	–	–	The sample contains <i>E. faecium</i> DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains <i>E. faecium</i> DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains <i>M. curtisii</i> DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains <i>M. curtisii</i> DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains <i>P. bivia</i> DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains <i>P. bivia</i> DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains <i>S. agalactiae</i> DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains <i>S. agalactiae</i> DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains <i>E. faecium</i> , <i>M. curtisii</i> , <i>P. bivia</i> , <i>S. agalactiae</i> DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains <i>E. faecium</i> , <i>M. curtisii</i> , <i>P. bivia</i> , <i>S. agalactiae</i> DNA and human RPP30 DNA.

## VERIFICATION EXPERIMENTS

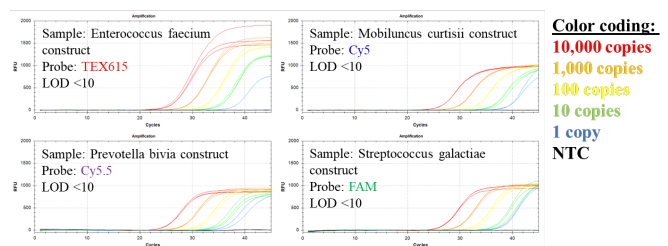
The Women’s Health 1 assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from *E. faecium*, *M. curtisii*, *P. bivia*, *S. agalactiae*, and human RPP30 DNA, which serves as a positive extraction-control assay.

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  $1 \times 10^4$  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.

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 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 bacterial types and are also compatible with RPP30\_DNA positive control primers. Verifications of the four 2-plex assays (pathogen + control assays) (data not shown) indicate that human genomic DNA matrix doesn’t affect detection of the pathogen DNA.

## CONTACT US

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

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## NOTES

<sup>1</sup> TEX615™ is a trademark of Thermo Fisher Scientific.

<sup>2</sup> BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

<sup>3</sup> Cy5™, a trademark of GE Healthcare.

<sup>4</sup> Cy5.5™, a trademark of Amersham Biosciences Corp

<sup>5</sup> FAM™ (Carboxyfluorescein), a trademark of Life Technologies, Inc

<sup>6</sup> BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

<sup>7</sup> HEX™ (Hexachloro-fluorescein), a trademark of Applied Biosystems Corp.

<sup>8</sup> “TaqMan” is a trademark of Roche Molecular Systems, Inc.