

ASSAY NAME: BFRA-D-QS

Quantity: 100 x 20µL PCR reactions

2-plex assay: *Bacteroides fragilis* and human RPP30 DNA

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

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

SCOPE OF THIS PRODUCT INFORMATION SHEET (PIS):

The oligonucleotide recipes are optimized for each instrument (QuantStudio, BioRad). The verification data presented in this PIS were performed using PNP-BFRA-D-QS-100 on a QuantStudio 7 Flex. 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 BFRA kit are provided in Tube 1 as a 20X concentrated working solution that detects the *B. fragilis* and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
<i>Bacteroides fragilis</i>	FAM	BHQ-1	1, 2
RPP30-DNA control	TAMRA	BHQ-2	3, 4

The probes are designed as TaqMan⁵ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

ASSAY HANDLING

The BFRA assay is shipped on ice, but 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.

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: Primer/Probe mix (20X) for *Bacteroides fragilis* and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments of *Bacteroides fragilis* and human RPP30DNA.

Tube 3: 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

Perform nucleic acid extraction/purification (recommended).

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

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

Notes: To improve assay sensitivity, up to 9 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.

A PCR protocol was used for verification on a QuantStudio™ 7 Flex 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 22 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, C_q. (C_q is preferred over C_t). Each fluorescence channel with a C_q < 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.

<i>B. fragilis</i> FAM™	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 <i>B. fragilis</i> DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains <i>B. fragilis</i> DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The BFRA kit validation was carried out as a 2-plex assay, which simultaneously detects DNA of *B. fragilis* 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 1×10⁴ 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**.

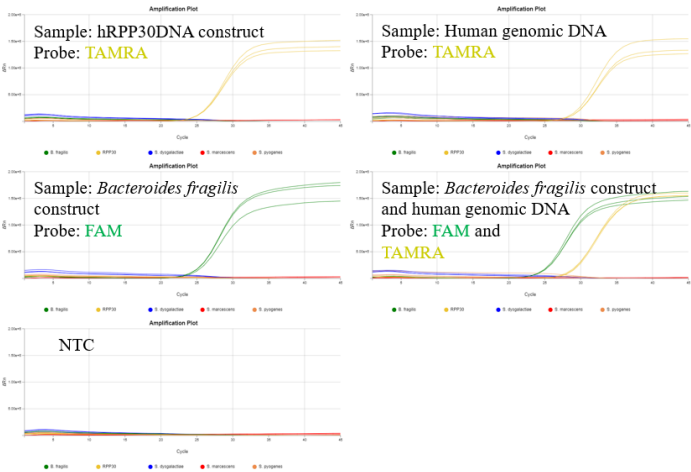


Figure 1: Verification experiments with single and double targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

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) <10 copies/reaction.

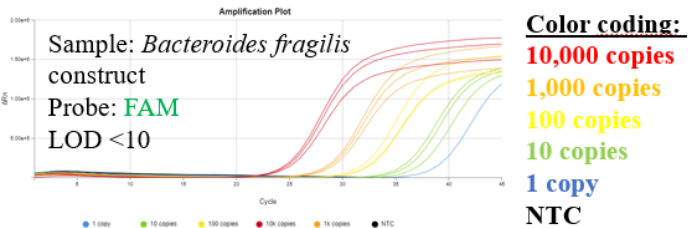


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicates that the *B. fragilis* primers and probe are compatible with DNAs RPP30 DNA positive control primers and probe in the human genomic DNA matrix.

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, Inc
- ² BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- ³ TAMRA™ (Carboxyltetramethylrhodamine), a trademark of AppleraCorp
- ⁴ BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- ⁵ “TaqMan” is a trademark of Roche Molecular Systems, Inc.