PCRassays.com

ASSAY NAME: BRP7_QS
(Bacterial Respiratory Panel 7 for QuantStudio)

Quantity: 100 x 20µL PCR reactions

5 color assay: *Bordetella pertussis, Bordetella parapertussis, Klebsiella pneumoniae*, *Mycoplasma pneumoniae, Streptococcus pneumoniae,* and human RPP30 DNA

SKU #: BUN-BRP7-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 (QuantStudio, BioRad). The verification data presented in this PIS were performed using BUN-BRP7-D-QS-100 on a QuantStudio™ 7 Flex Real-Time System. 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 BRP7_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects 5 pathogens and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
B. pertussis and B. parapertussis	**FAM**	BHQ-1	1,2
K. pneumoniae	**HEX**	BHQ-1	3
RPP30-DNA control	**TAMRA**	BHQ-2	4, 5
S. pneumoniae	**TEX615**	BHQ-2	6
M. pneumoniae	**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.

ASSAY HANDLING

The BRP7_QS 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.

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 *B. pertussis, B. parapertussis*, *K. pneumoniae*, *M. pneumoniae*, *S. pneumoniae*, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for *B. pertussis, B. parapertussis*, *K. pneumoniae*, *M. pneumoniae*, *S. pneumoniae*, and hRPP30DNA.

Tube 3: 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

Perform nucleic acid extraction/purification (recommended). Since some of the bacteria in this assay are Gram+, it is important to use an extraction procedure with an appropriate cell wall lysis agent.

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 (Molecular Biology Grade)	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.

A PCR protocol was used for verification on a QuantStudio™ 7 Flex 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 22 seconds
4	Plate Read
5	Go to Step 2, repeat 44× more

RESULT INTERPRETATION

After running the qPCR reaction, use the instrument software to determine the quantification cycle, Cq (or use Ct if your instrument does not have the capability to compute a Cq). Fluorescence channels with a Cq < 38 cycles, and final RFU >Threshold is considered "positive" or "+" in the Table below. The "Threshold" value for calling a PCR positive is dependent on the instrument model, well size, and sample volume; thus the user must determine the threshold that is appropriate for their method. For our QuantStudio 7 Flex with 96 well plate with 100 µL wells and 20 µL reaction volume, the average RFU was approximately 1,000,000 and we used a threshold of 200,000 for calling positives.

B. pertussis/ B. para-pertussis FAM™	*K. pneumo niae* HEX™	hRPP30 TAMRA ™	*S. pneumoniae* TEX615™	*M. pneu-moniae* Cy5™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	+	–	–	The sample does not contain pathogen DNA. The sample contains human RPP30 DNA.
+	–	–	–	–	The sample contains *B. pertussis* and/or *B. parapertussis* DNA. The sample may not contain human RPP30 DNA.
+	–	+	–	–	The sample contains *B. pertussis* and/or *B. parapertussis* DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *K. pneumoniae* DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains *K. pneumoniae* DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains *S. pneumoniae* DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	–	The sample contains *S. pneumoniae* DNA and *human* RPP30 DNA.
–	–	–	–	+	The sample contains *M. pneumoniae* DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains *M. pneumoniae* DNA and human RPP30 DNA.
+	+	–	+	+	The sample contains *B. pertussis* and/ or *B. parapertussis*, *K. pneumoniae*, *S. pneumoniae*, *M. pneumoniae* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *B. pertussis* and/or *B. parapertussis*, *K. pneumoniae*, *S. pneumoniae*, *M. pneumoniae* DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The BRP7_QS assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from *B. pertussis, B. parapertussis, K. pneumoniae, M. pneumoniae, S. pneumoniae,* 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 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.

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.

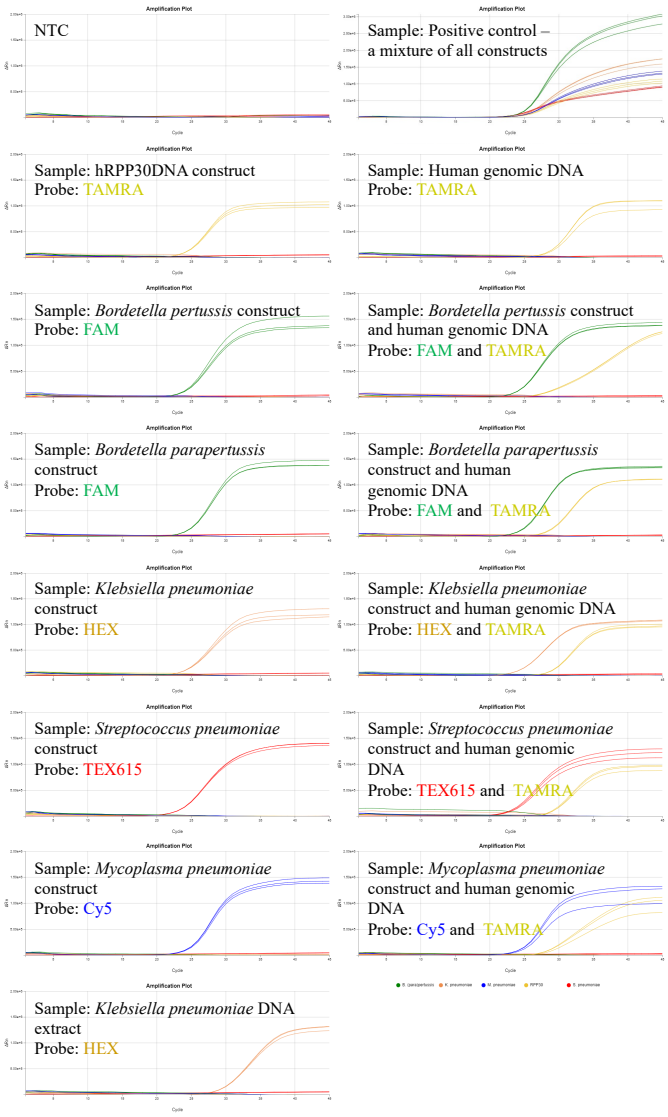

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

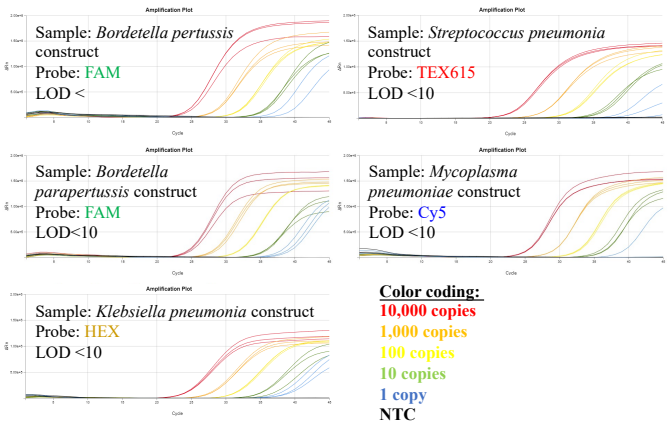

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

NOTES

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

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

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

[4] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Cor.

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

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

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