Kit: Bacterial-URP4
Quantity: 100 x 20µL PCR reactions

5-plex assay: *Bordetella pertussis*, *Klebsiella aerogenes*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and human RPP30 DNA
Gene: A hypothetical protein (B. per), sugar porter family MFS transporter protein (K. aer), NA+/H+ antiporter Mnh2 protein (S. epi), an intergenic region (K. pne)
SKU#: PNP-BRP4-D-100

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

Kit contents:

Tube 1: 5X Primer/Probe mix for *B. pertussis*, *K. aerogenes*, *S. epidermidis*, *K. pneumoniae*, and hRPP30DNA

Tube 2: 5000 copies/µl Positive controls of synthetic 500 bp DNA for all targets and human RPP30DNA.

CONTENTS

The Bacterial-URP4 kit contains a mixture of primers/probes targeting a hypothetical protein in the *Bordetella pertussis* genome, sugar porter family MFS transporter protein (Protein ID: QEU20225.1) in the *Klebsiella aerogenes* genome, NA+/H+ antiporter Mnh2 protein, subunits G and F (Protein IDs: UUY59018.1, UUY59019.1) in the *Staphylococcus epidermidis* genome, and an intergenic region in the *Klebsiella pneumoniae* genome. The primers and probes in Tube 1 are provided as a 5X concentrated working solution. The fluorophore of the *B. pertussis* probe is **FAM™** and the quencher is BHQ-1™.[1,2] The fluorophore of the K. aerogenes probe is **TEX615™** and the quencher is BHQ-2™.[3,4] The fluorophore of the S. epidermidis probe is **Cy5™** and the quencher is BHQ-2™.[5] The fluorophore of the *K. pneumoniae* probe is **Cy5.5™** and the quencher is BHQ-2™.[6] The same mix also contains primers/probe targeting human RPP30 DNA Intron I (5X concentrated) as a PCR positive control assay for human samples. The fluorophore of the hRPP30DNA probe is **HEX™** and the quencher is BHQ-1™.[7] The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences, Cat number: ITMP-MM-2500)

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of all targets and RPP30 DNA is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.

KIT HANDLING AND CONTAMINATION

The Bacterial-URP4 kit is shipped at ambient temperature, and should be stored at approximately -20°C. The kit 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.

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (5X)	4
Sample	2
Water	4

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR MasterMix, from Empirical Biosciences. In a reaction with the double positive control, 2 µL of the solution from Tube 2 should be added.

A PCR protocol was used in-house for pre-validation 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 44xmore

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 >200 is considered "positive" or "+" in the Table.

B. pertussis FAM™	*K. aerogenes* TEX615™	*S. epidermidis* Cy5™	*K. pneumoniae* Cy5.5™	RPP 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 *B. pertussis* DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains *B. pertussis* DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *K. aerogenes* DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains *K. aerogenes* DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains *S. epidermidis* DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains *S. epidermidis* 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 *B. pertussis*, *K. aerogenes*, *S. epidermidis*, and *K. pneumoniae* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *B. pertussis*, *K. aerogenes*, *S. epidermidis*, and *K. pneumoniae* DNA. and human RPP30 DNA.

PRE-VALIDATION EXPERIMENTS

The Bacterial-URP4 kit validation was carried out as a 5-plex assay, which simultaneously detects DNA from *B. pertussis*, *K. aerogenes*, *S. epidermidis*, *K. 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 validation experiments contained 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genomes and the human RPP30 DNA gene. The results of these experiments are shown in **Figure 1** below:

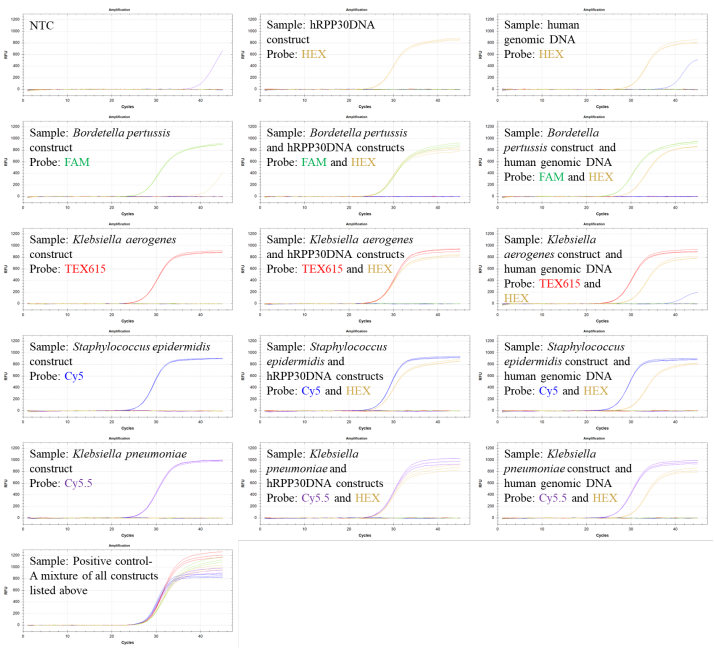

Figure 1: Validation experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. A small amount of Cy5 signal is shown in the NTC, the Cq >38 so it is considered negative. The **FAM** probe detects *B. pertussis* DNA. The **TEX615** probe detects *K. aerogenes* DNA. The **Cy5** probe detects *S. epidermidis* DNA. The **Cy5.5** probe detects *K. pneumoniae*. The **HEX** probe detects human RPP30 DNA.

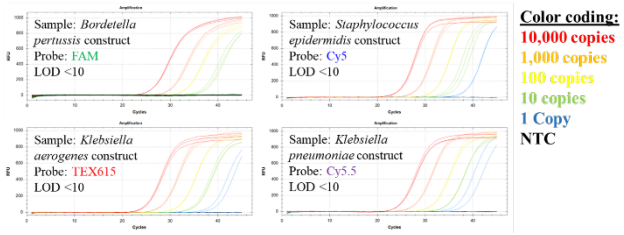

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the *B. pertussis, K. aerogenes, S. epidermidis, K. pneumoniae* are compatible with DNAS RPP30 DNA positive control primers and probe in the human genomic DNA matrix.

The limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate. For dilution series only target construct was added. The results show a limit of detection (LOD) <10 copies/reaction for each target.

CONTACT US

For assistance, please contact DNA Software using the link: https://dnasoft.jira.com/servicedesk/customer/portals

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

Phone: (734) 222-9080

NOTES

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

[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™, a trademark of Amersham Biosciences Corp

[7] HEX™ (Hexachloro-fluorescein), a trademark of Applera Corp.

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