Kit: Bacterial-URP3
Quantity: 100 x 20µL PCR reactions

5-plex assay: *Legionella pneumophila*, *Haemophilus influenzae*, *Streptococcus pyogenes*, *Mycoplasma pneumoniae* and human RPP30 DNA
Genes: Intergenic region (L. pne), phosphate acetyltransferase protein (H. inf), 16S rRNA methyltransferase protein RsmC (S. pyo), 30S ribosomal protein S17 (M. pne)

SKU#: PNP-BRP3-D-100

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

Kit contents:

Tube 1: 5X Primer/Probe mix for *L. pneumophila*, *H. influenzae, S. pyogenes*, *M. pneumoniae,* and hRPP30DNA.

Tube 2: 5000 copies/µl Positive controls of synthetic DNA for all targets and hRPP30.

CONTENTS

The Bacterial-URP3 kit contains a mixture of primers/probes targeting an intergenic region (i.e. no gene known in the region) in the *Legionella pneumophila* genome, the gene of the phosphate acetyltransferase protein (Protein ID: BCB67200.1) in the *Haemophilus influenzae* genome, the 16S rRNA methyltransferase protein RsmC (Protein ID: QJC39666.1) in the *Streptococcus pyogenes* genome, and the gene of a 30S ribosomal protein S17 (Protein ID: QHR23375.1) in the *Mycoplasma pneumonia* genome. The primers and probes in Tube 1 are provided as a 5X concentrated working solution. The fluorophore of the *L. pneumophila* probe is **TEX615™** and the quencher is BHQ-2™.[1,2] The fluorophore of the *H. influenzae* probe is **Cy5™** and the quencher is BHQ-2™.[3] The fluorophore of the *S. pyogenes* probe is **Cy5.5™** and the quencher is BHQ-2™.[4] The fluorophore of the *M. pneumoniae* probe is **FAM™** and the quencher is BHQ-1™.[5,6] The same mix also contains primers/probe targeting human RPP30 mRNA (5X concentrated) as a RT-PCR positive control assay for human samples. The fluorophore of the RPP30DNA 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 RT-qPCR Mastermix, from Empirical Biosciences, Cat number: RTQAK-200)

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of all targets and RPP30 RNA 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-URP3 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 clean hood.

EXPERIMENTAL

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

Component	Volume (µL)
InhibiTaq enzyme mastermix (2X)	10
Primer/Probe mix (20X)	4
Sample	2
Water	4

Note: The composition of this reaction is calculated based on the user manual of RTQAK RT-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 3, repeat 44x more

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 below.

Legionella pneumophila TEX615™	*Haemo. influenzae* Cy5™	*Strep. pyogenes* Cy5.5™	*Myco. pneumoniae* 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 *L. pneumophila* DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains *L. pneumophila* DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains *H. influenzae* DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains *H. influenzae* DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains *S. pyogenes* DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains *S. pyogenes* 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 *L. pneumophila*, *H. influenzae*, *S. pyogenes*, *M. pneumoniae* DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains *L. pneumophila*, *H. influenzae*, *S. pyogenes*, *M. pneumoniae* DNA and human RPP30 DNA.

PRE-VALIDATION EXPERIMENTS

The Bacterial-URP3 kit validation was carried out as a multiplexed assay, which simultaneously detects DNA of *L. pneumophila, H. influenzae, S. pyogenes, M. 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, and human genomic DNA. The results of these experiments are shown in **Figure 1** below:

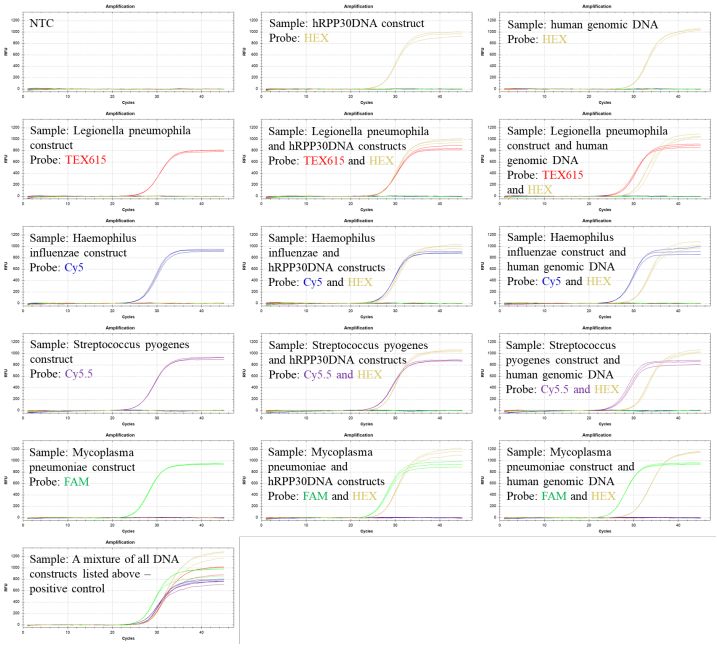

Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The TEX615 probe detects *L. pneumophila*. The Cy5 probe detects *H. influenzae*. The Cy5.5 probe detects *S. pyogenes*. The FAM probe detects *M. pneumoniae*. The HEX probe detects human RPP30 DNA.

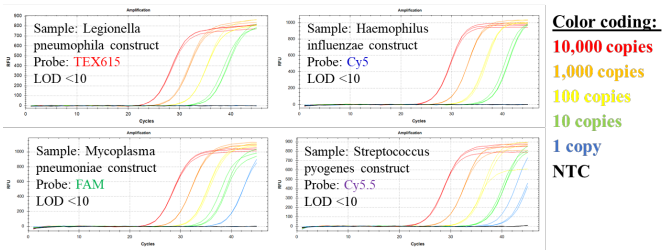

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicates that the *L. pneumophila, H. influenzae, S. pyogenes, M. pneumoniae* primers and probe 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

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NOTES

[1] TEX615™ is a trademark of Thermo Fisher Scientific.

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

[3] Cy5™, a trademark of GE Healthcare.

[4] Cy5.5™, a trademark of Amersham Biosciences Corp

5 FAM™ (Carboxyfluorescein), a trademark of Life Technologies, Inc

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

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

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