Assay: S.pneumo-RPP30DNA
Quantity: 100 x 20µL PCR reactions
Detects: *Streptococcus pneumoniae* and human genomic RPP30DNA
Gene: A hypothetical protein (AAL00743.1)
(RUO). Research Use Only. Not for use in Diagnostic Procedures.
Cat #: STRPNE-RPP30DNA-TC-0041

Kit contents:
Tube 1: 20X primers/probe specific for *Streptococcus pneumoniae*.
20X primers/probe specific for human RPP30DNA Intron I.

INTRODUCTION

The assay for S.pneumo-RPP30DNA is a real-time polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from *Streptococcus pneumoniae* with FAM™ (Carboxyfluorescein, a trademark of Life Technologies, Inc). The *Streptococcus pneumoniae* assay detects the gene for a hypothetical protein (Protein ID: AAL00743.1). This kit is for research use only and should not be used for diagnostic procedures.

CONTENTS

A mix of primers/probe targeting the region coding for a hypothetical protein (AAL00743.1) in the *Streptococcus pneumoniae* genome is provided in a tube as a 20X concentrated working solution. The fluorophore of the probe for *Streptococcus pneumoniae* is FAM™ and the quencher is BHQ-1™ (Black Hole Quencher, a trademark of Biosearch Technologies, Inc.). The same mix also contains primers/probe targeting human RPP30DNA Intron I (20X concentrated) as a PCR positive control assay for human samples. The fluorophore of the probe is HEX™ (Hexachloro-fluorescein, a trademark of Applera Corp.), and the quencher is BHQ-1™.

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 assay for S.pnemo-RPP30DNA is shipped at ambient temperature, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed.

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 mastermix (2×)	10
Primer and probe mix (20×)	1
Sample	2
Water	7

Note: The composition of this reaction is calculated based on the user manual of InhibiTaq PLUS qPCR Master Mix, from Empirical Biosciences. The volume of water should be adjusted accordingly if the user's reaction preparation is different from the recommended preparation method.

A PCR protocol was used at DNA Software, Inc. 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 30 seconds
4	Plate Read
5	Go to Step 2, repeat 44x more
6	(optional) Incubate @55 °C for 3 minutes

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.

Streptococcus pneumoniae (FAM™)	RPP30DNA (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample doesn't contain *Streptococcus pneumoniae* DNA.
+	–	The sample contains *Streptococcus pneumoniae* DNA. The sample may not contain human RPP30DNA Intron I.
+	+	The sample contains *Streptococcus pneumoniae* DNA and human RPP30DNA Intron I.

PRE-VALIDATION DATA

The assay validation was carried out as a 2-plex assay, S.pneumo-RPP30DNA, to detect DNA from *Streptococcus pneumoniae* and DNA from the human RPP30DNA gene, which serves as a control assay to detect human DNA.

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 DNA constructs (from Twist Biosciences) harboring the regions of interest from *Streptococcus pneumoniae* genome and the RPP30DNA gene. The results of these experiments are shown in **Figure 1** below:

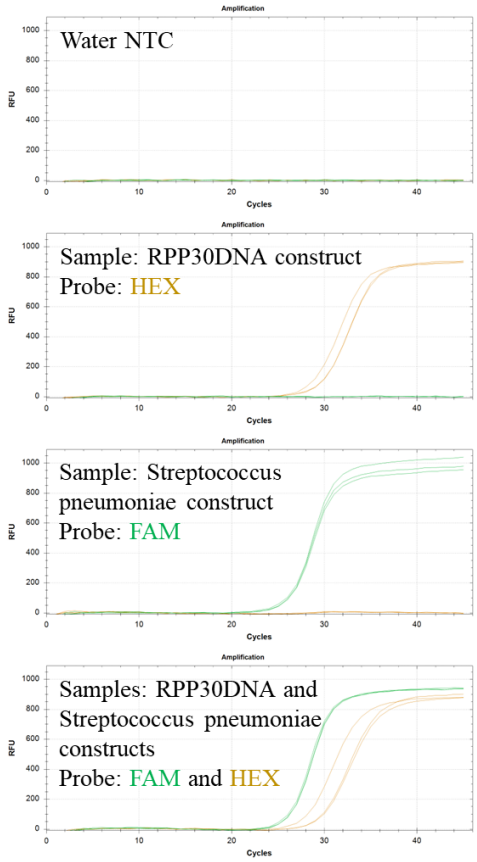
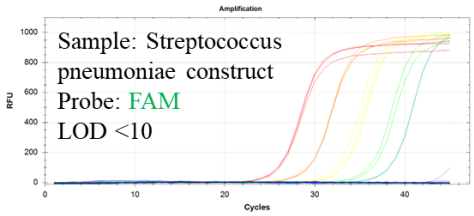


Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) (DNA construct(s)) is present, indicating that the amplification is specific. The **FAM** probe detects *Streptococcus pneumoniae* construct (DNA). The **HEX** probe detects RPP30DNA construct (human genomic DNA).



Color coding:
10,000 copies
1,000 copies
100 copies
10 copies
1 copy
NTC

Figure 2: Serial dilution experiments show LOD <10 molecules for *Streptococcus pneumoniae* construct.

Conclusion: The data in **Figure 1** indicate that the Streptococcus pneumoniae primers and probe are compatible with DNAS RPP30DNA positive control primers and probes in a 2-plex application to detect *Streptococcus pneumoniae* in the matrix of human sample extract.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only the Streptococcus pneumoniae construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

CONTACT US

For further assistance, please contact DNA Software using the link: https://www.dnasoftware.com/contact/

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