PCRassays.com

ASSAY NAME: TET_QS (Tetracycline AMR Panel for QuantStudio)

Quantity: 100 x 20µL PCR reactions

3-plex assay: tetB, tetM and human RPP30 DNA

SKU: BUN-TET-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 (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using PNP-TETFOS-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 need to use a different qPCR instrument.

CONTENTS

The primers and probes in the TET assay are provided in Tube 1 as a 5X concentrated working solution that detects tetB/M and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
tetB/M	FAM	BHQ-1	1, 2
RPP30-DNA control	TAMRA	BHQ-1	3, 4

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

Table of AMR Genes, Drugs and Organisms:

AMR Gene	Drugs	Organisms
tet(B)	tetracycline, doxycycline and minocycline, and oxytetracycline, but not tigecycline	*Haemophilus parasuis, Pasteurella aerogenes, Actinobacillus pleuropneumoniae, Escherichia coli, Neisseria meningitidis, Pasteurella multocida, Photobacterium spp., Pseudomonas spp., Shigella flexneri*
tet(M)	tetracycline, doxycycline and minocycline, and oxytetracycline, but not tigecycline	*Acinetobacter junii, Clostridium perfringens, Clostridium septicum, Enterococcus faecalis, Enterococcus faecium, Erysipelothrix rhusiopathiae, Escherichia coli, Gardnerella vaginalis, Klebsiella pneumoniae, Lactobacillus plantarum, Neisseria meningitidis, Peptoclostridium difficile, Staphylococcus aureus, Streptococcus cristatus, Streptococcus mitis, Streptococcus oralis, Streptococcus parauberis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus suis, Ureaplasma urealyticum, Vibrio sp.*

ASSAY HANDLING AND CONTAMINATION

The TET assay is shipped on ice, and 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.

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

Assay contents:

Tube 1: Primer/Probe mix (5X) for tetB/M and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: Synthetic 500 bp DNA fragments of tetB/M 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 (5X)	4
Sample or Positive Control	2
Water	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 (i.e., the "sample").

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, C_q (or use C_T if your instrument does not have the capability to compute a C_q). Fluorescence channels with a C_q < 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 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 or "+" in the Table below.

tetB/M FAM™	RPP30 TAMRA™	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 tet DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains tet DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The TET assay verification was previously verified as part of the study of a 3-plex assay, which simultaneously detects DNA from tetB, tetM, tetS, fosA, and human RPP30 DNA, which serves as a positive extraction-control assay. Data from tetB, tetM, and control channel show the performance expected for the TET 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 synthetic 500 bp DNA constructs (from Twist Biosciences) harboring the regions of interest from the AMR genomes, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 2-plex specifically detects the different AMR genes.

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.

NOTES

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

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

[3] TAMRA™ (Carboxyltetramethylrhodamine) trademark of Applera Corp.

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

[5] TaqMan™ is a trademark of Roche Diagnostics, Inc.

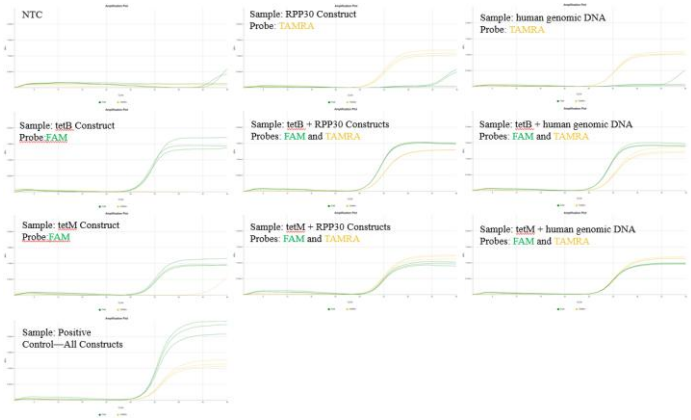

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

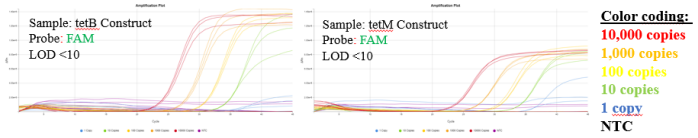

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 2-plex primers and probes specifically detect and differentiate the AMR genes and are also compatible with RPP30_DNA positive control primers. Human genomic DNA doesn't interfere with the detection of the AMR genes.

CONTACT US

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

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