PCRassays.com

ASSAY NAME: TMQS2_QS (Trimethoprim, Macrolide, Quinolone, sulfonamide AMR Panel)

Quantity: 100 x 20µL PCR reactions

5-color assay: ermA, ermB, ermC, DfrA1, DfrA5, qnrA, qnrS, sul1, sul2, and human RPP30 DNA

SKU #: PNP-TMQS2-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 (QuantStudio, BioRad). The verification data presented in this PIS were performed with PNP-TMQS2-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 TMQS2_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 pathogens and a human control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
ermA, ermB, ermC	FAM	BHQ-1	1, 2
DfrA1, DfrA5	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4, 5
qnrA/S	TEX615	BHQ-2	6
sul1, sul2	Cy5	BHQ-2	7

The primer and probes for each assay detect multiple alleles of each AMR gene (see Table bottom right) and confer resistance to multiple drugs and in different organisms (see Table below).

Table of AMR Genes, Drugs and Organisms:

AMR Gene	Drugs	Organisms
DfrA1	Trimetoprim	*Salmonella enterica, Vibrio cholerae*
DfrA5	Trimetoprim	*Acinetobacter baumannii, Escherichia coli*
erm(A)	Macrolides, Lincosamides, And Streptogramin B, Mlsb, Erythromycin, Clindamycin, Quinupristin/dalfopristin	*Staphylococcus aureus, Streptococcus pyogenes*
erm(B)	Macrolides, Lincosamides, And Streptogramin B, Mlsb, Erythromycin, Clindamycin, Quinupristin/dalfopristin	*Enterococcus faecalis, Enterococcus faecium, Lactobacillus reuteri, Peptoclostridium difficile, Staphylococcus intermedius, Streptococcus agalactiae, Streptococcus pyogenes*
erm(C)	Macrolides, Lincosamides, And Streptogramin B, Mlsb, Erythromycin, Clindamycin, Quinupristin/dalfopristin	Bacillus subtilis, Lactobacillus reuteri, Staphylococcus aureus, Staphylococcus chromogenes, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus hyicus, Staphylococcus saprophyticus Staphylococcus simulans
qnrA	Quinolone, Fluoroquinolone, Ciprofloxacin, Levofloxacin, Norfloxacin, Gatifloxacin, Cefpodoxime, Cefotaxime, Enrofloxacin, Trimethoprim- Sulfamethoxazole and Penicillin	*Enterobacter cloacae, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Shewanella algae*
qnrS	Quinolone, Fluoroquinolone, Ciprofloxacin, Levofloxacin, Norfloxacin, Gatifloxacin, Cefpodoxime, Cefotaxime, Enrofloxacin, Trimethoprim- Sulfamethoxazole And Penicillin	*Aeromonas hydrophila, Aeromonas sobria, Enterobacter hormaechei, Escherichia coli, Klebsiella pneumoniae, Salmonella enterica, Shigella flexneri*
sul1	Sulfonamide	*Acinetobacter baumannii, Aeromonas hydrophila, Aeromonas salmonicida, Corynebacterium diphtheriae, Corynebacterium striatum, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, Salmonella enterica, Shigella flexneri*
sul2	Sulfonamide	*Haemophilus parasuis, Actinobacillus pleuropneumoniae, Actinobacillus porcitonsillarum, Aliivibrio salmonicida, Bibersteinia trehalosi, Mannheimia varigena, Pasteurella multocida, Pasteurella multocida, Salmonella enterica, Shigella sonnei, Vibrio cholerae*

ASSAY CONTENTS:

Tube 1: 5X Primer/Probe mix for DfrA1/5, ermA/B/C, qnrA/S, sul1/2, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for DfrA1/5, ermA/B/C, qnrA/S, sul1/2, 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.

ASSAY HANDLING

The TMQS2_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.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

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

Table of Alleles covered by each PCR assay:

Assay	Alleles Covered by PCRassays.com Test
DfrA1	DfrA1
DfrA5 group	DfrA5, DfrA30
erm(A)	erm(A)
erm(B)	erm(B)
erm(C)	erm(C)
QnrA group	QnrA1, QnrA2, QnrA3, QnrA4, QnrA5, QnrA6, QnrA7, QnrA9, QnrA10, QnrA11, QnrA12, QnrA13
QnrS group	QnrS1, QnrS2, QnrS4, QnrS5, QnrS6, QnrS7, QnrS8, QnrS9, QnrS10, QnrS11, QnrS12, QnrS13, QnrS14, QnrS15
sul1	sul1
sul2	sul2

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). Due to low level contamination (<1 molecule per reaction on average) of sul1 and sul2 genes in the enzyme mastermix due to expression in *E. coli*, 10-30% of no template control (NTC) wells display Cy5 signal with Cq of 37-40 (note that qPCR mastermix from other vendors does not have this impurity). See Figure 1 caption for further description. Thus, we consider true positives or "+" in the table below for reactions with Cq <34 cycles for sul1/2 and Cq <38 cycles for all other targets. In addition, a "positive" requires an RFU >Threshold is considered "positive" or "+" in the Table below. The RFU "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.

ermA/B/C FAM™	DfrA1/5 HEX™	RPP30 TAMRA™	qnrA/S TEX615™	sul1/2 Cy5™	**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 ermA/B/C DNA. The sample may not contain human RPP30 DNA.
+	–	+	–	–	The sample contains ermA/B/C DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains DfrA1/5 DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains DfrA1/5 DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains qnrA/S DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	–	The sample contains qnrA/S DNA and human RPP30 DNA.
–	–	–	–	+	The sample contains Sul1/2 DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains Sul1/2 DNA and human RPP30 DNA.
+	+	–	+	+	The sample contains ermA/B/C, DfrA1/5 , qnrA/S, and Sul1/2 DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains ermA/B/C, DfrA1/5, qnrA/S, Sul1/2 DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The TMQS2_QS assay verification was carried out as a 5-color assay, which simultaneously detects DNA from DfrA1/5, ermA/B/C, qnrA/S, sul1/2, 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-color specifically detects the different AMR genes in the human genomic DNA matrix.

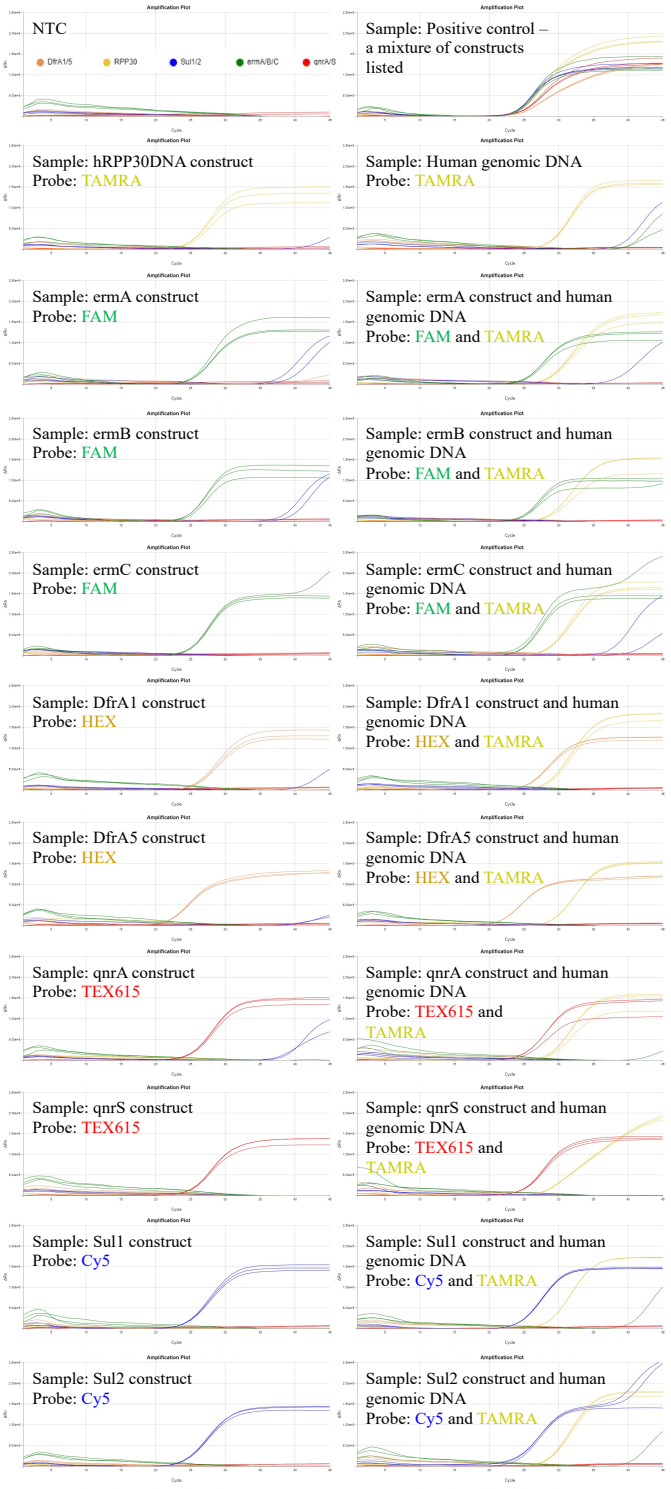

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. Note the presence of late Cy5 signal in some of the panels. This is due to sul1 and sul2 gene contamination in the enzyme mastermix. Thus we recommend using threshold of Cq < 34 for calling positives of sul1 and sul2. The true positives for sul1 (not observed in this particular experiment) will sometimes show an additional transition which is due to contamination of sul2. The true positives for sul2 (bottom right panel) will sometimes show an additional transition which is due to contamination of sul1.

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 LOD for ermA/B/C, DfrA1/5, and qnrA1/5 are all <10 molecules. Due to the contamination of sul1 and sul2 genes in the enzyme mastermix, we conservatively estimate that the LOD for sul1 and sul2 is ~20 molecules per reaction corresponding to a Cq of 34.

Conclusion: The data in **Figure 1** indicate that the 5-color primers and probes specifically detect and differentiate the AMR genes and are also compatible with RPP30_DNA positive control primers.

NOTES

[1] FAM™ (Carboxyfluorescein) is a trademark of Life Technologies, Inc
[2] BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
[3] HEX™ (Hexachloro-fluorescein) is a trademark of Applera Corp.
[4] TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Corp.
[5] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
[6] TEX615™ is a trademark of Thermo Fisher Scientific.
[7] Cy5™ is a trademark of GE Healthcare
[8] "TaqMan" is a trademark of Roche Molecular Systems, Inc.

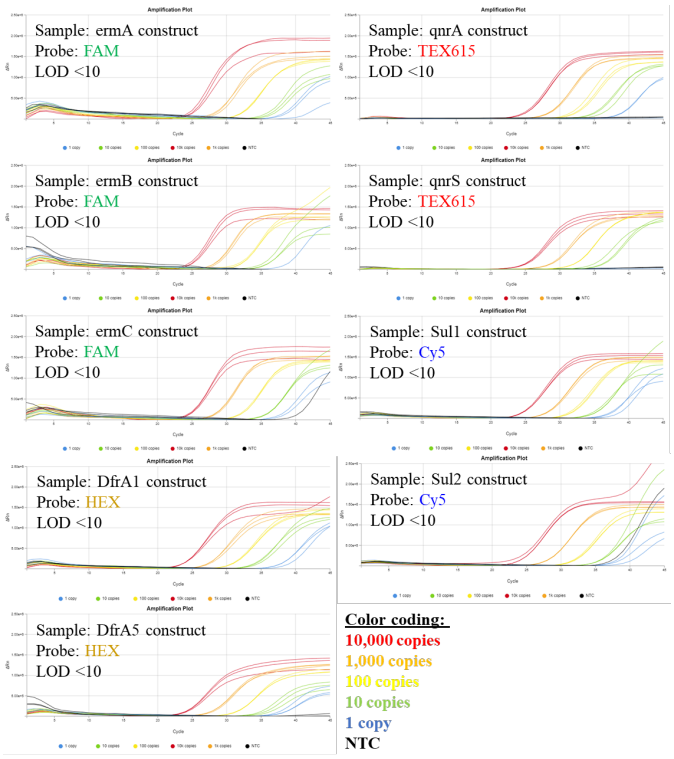

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

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

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