

ASSAY NAME: AMR3A_BR384
(AMR Panel 3A for Bio-Rad 384 or 96)

Quantity: 100 x 20µL PCR reactions

3-color (7-plex) assay: tetB, tetM, tetS, OXA-23, OXA-48, OXA-58, and human RPP30 DNA

SKU: BUN-AMR3A-D-BR384-100 (Bio-Rad)

(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 (Bio-Rad, QuantStudio). The verification data presented in this PIS were performed using kit BUN-AMR3A-D-BR384-100 on a Bio-Rad CFX96 instrument using a white-bottomed plate. This kit is compatible with both CFX-96 and CFX-384 instruments. The performance of the other SKUs on their corresponding instrument should be similar. Contact PCRassays.com if you are planning to use a different qPCR instrument.

CONTENTS

The primers and probes in the AMR3A assay are provided in Tube 1 as a 5X concentrated working solution that detects the 6 AMR genes and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
tetB/M/S	FAM	BHQ-1	1, 2
RPP30-DNA control	HEX	BHQ-1	3
OXA-23/48/58	TEX615	BHQ-2	4, 5

The probes are designed as TaqMan⁷ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

Table of alleles covered by each PCR assay

Assay	Alleles Covered by Each PCR Assay
tet(B) group	Tet(B)
tet(M) group	Tet(M)
tet(S) group	Tet(S)
OXA-23 group	OXA-23, OXA-27, OXA-49, OXA-73, OXA-105, OXA-133, OXA-146, OXA-165, OXA-166, OXA-167, OXA-168, OXA-169, OXA-170, OXA-171, OXA-225, OXA-239, OXA-366, OXA-398, OXA-422, OXA-423, OXA-435, OXA-440, OXA-482, OXA-483, OXA-565, OXA-657, OXA-806, OXA-807, OXA-808, OXA-809, OXA-810, OXA-811, OXA-812, OXA-813, OXA-814, OXA-815, OXA-816, OXA-817, OXA-818, OXA-893, OXA-911, OXA-966, OXA-967, OXA-968, OXA-969, OXA-1095, OXA-1216, OXA-1223
OXA-48 group	OXA-48, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245, OXA-247, OXA-252, OXA-370, OXA-405, OXA-416, OXA-438, OXA-439, OXA-484, OXA-505, OXA-514, OXA-515, OXA-517, OXA-519, OXA-538, OXA-546, OXA-547, OXA-566, OXA-567, OXA-788, OXA-793, OXA-833, OXA-894, OXA-918, OXA-920, OXA-922, OXA-923, OXA-924, OXA-929, OXA-933, OXA-934, OXA-1012, OXA-1038, OXA-1039, OXA-1055, OXA-1119, OXA-1146, OXA-1181, OXA-1200, OXA-1201, OXA-1205, OXA-1207, OXA-1211, OXA-1212, OXA-1213
OXA-58 group	OXA-58, OXA-96, OXA-97, OXA-164, OXA-397, OXA-420, OXA-512, OXA-1178

Assay contents:

Tube 1: 5X Primer/Probe mix for tetB/M/S, OXA-23/48/58, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments of tetB, OXA-48, 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.



ASSAY HANDLING

The AMR3A kit 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.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended). Set up your PCR 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 (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. For 10 µL reactions, divide all of the amounts above by a factor of 2.

A PCR protocol was used for verification on a Bio-Rad CFX96 system using white plates, 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 20 seconds*
4	Plate Read
5	Go to Step 2, repeat 44x more

* A 15 sec. extension time may be used for Bio-Rad CFX96 inst.

Table of AMR genes, drugs and organisms:

AMR Gene	Drugs	Organisms
tet(B)	tetracycline, doxycycline and minocycline, and oxytetracycline, but not tigecycline	<i>Haemophilus parasuis</i> , <i>Pasteurella aerogenes</i> , <i>Actinobacillus pleuropneumoniae</i> , <i>Escherichia coli</i> , <i>Neisseria meningitidis</i> , <i>Pasteurella multocida</i> , <i>Photobacterium spp.</i> , <i>Pseudomonas spp.</i> , <i>Shigella flexneri</i>
tet(M)	tetracycline, doxycycline and minocycline, and oxytetracycline, but not tigecycline	<i>Acinetobacter junii</i> , <i>Clostridium perfringens</i> , <i>Clostridium septicum</i> , <i>Enterococcus faecalis</i> , <i>Enterococcus faecium</i> , <i>Erysipelothrix rhusiopathiae</i> , <i>Escherichia coli</i> , <i>Gardnerella vaginalis</i> , <i>Klebsiella pneumoniae</i> , <i>Lactobacillus plantarum</i> , <i>Neisseria meningitidis</i> , <i>Peptoclostridium difficile</i> , <i>Staphylococcus aureus</i> , <i>Streptococcus cristatus</i> , <i>Streptococcus mitis</i> , <i>Streptococcus oralis</i> , <i>Streptococcus parauberis</i> , <i>Streptococcus pneumoniae</i> , <i>Streptococcus pyogenes</i> , <i>Streptococcus suis</i> , <i>Ureaplasma urealyticum</i> , <i>Vibrio sp.</i>
tet(S)	tetracycline, doxycycline and minocycline, and oxytetracycline, but not tigecycline	<i>Streptococcus intermedius</i> , <i>Listeria monocytogenes</i> , <i>Streptococcus thermophilus</i>
OXA-23 group	Penicillin, Carbapenem	<i>Acinetobacter baumannii</i> , <i>Acinetobacter radiresistens</i> , <i>Klebsiella pneumoniae</i>
OXA-48 group	Penicillin, Carbapenem	<i>Citrobacter freundii</i> , <i>Enterobacter cloacae</i> , <i>Enterobacter kabeii</i> , <i>Escherichia coli</i> , <i>Klebsiella aerogenes</i> , <i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Providencia stuartii</i> , <i>Serratia marcescens</i> , <i>Shewanella xiamensis</i>
OXA-58 group	Penicillin, Carbapenem	<i>Acinetobacter baumannii</i>

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). PCR reactions are considered “positive” or “+” in the table below if the RFU > “threshold” and the C_q < 38 cycles. “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 our Bio-Rad CFX-96 with 200 μ L wells (with white-bottomed plate) and 20 μ L reaction volume, the average RFU was approximately 3,500, we used a threshold of 350 for calling positives or “+” in the Table below.

tetB/M/S FAM TM	OXA-23/48/58 TEX615 TM	RPP30 HEX TM	Recommended Interpretation
—	—	—	The PCR reaction failed. Please repeat the experiment.
—	—	+	The sample contains human RPP30 DNA. The sample doesn't contain pathogen DNA.
+	—	—	The sample contains tetB/M/S DNA. The sample may not contain human RPP30 DNA.
+	—	+	The sample contains tetB/M/S DNA and human RPP30 DNA.
—	+	—	The sample contains OXA-23/48/58 DNA. The sample may not contain human RPP30 DNA.
—	+	+	The sample contains OXA-23/48/58 DNA and human RPP30 DNA.
+	+	—	The sample contains tetB/M/S and OXA-23/48/58 DNA. The sample may not contain human RPP30 DNA.
+	+	+	The sample contains tetB/M/S, OXA-23/48/58 DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The AMR3A assay verification was carried out as a 7-plex assay, which simultaneously detects DNA from tetB, tetM, tetS, OXA-23, OXA-48, OXA-58, 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 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 7-plex specifically detects the different AMR genes in the human genomic DNA matrix.

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

Conclusion: The data in **Figure 1** indicate that the 7-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 pathogens.

NOTES

- ¹ FAMTM (Carboxyfluorescein), a trademark of Life Technologies Corp.
- ² BHQ-1TM (Black Hole Quencher) is a trademark of Biosearch Tech, Inc.
- ³ HEXTM (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.
- ⁴ TEX615TM is a trademark of Thermo Fisher Scientific.
- ⁵ BHQ-2TM (Black Hole Quencher) is a trademark of Biosearch Tech, Inc.
- ⁶ Cy5TM is a trademark of GE Healthcare.
- ⁷ TaqManTM is a trademark of Roche Diagnostics, Inc.

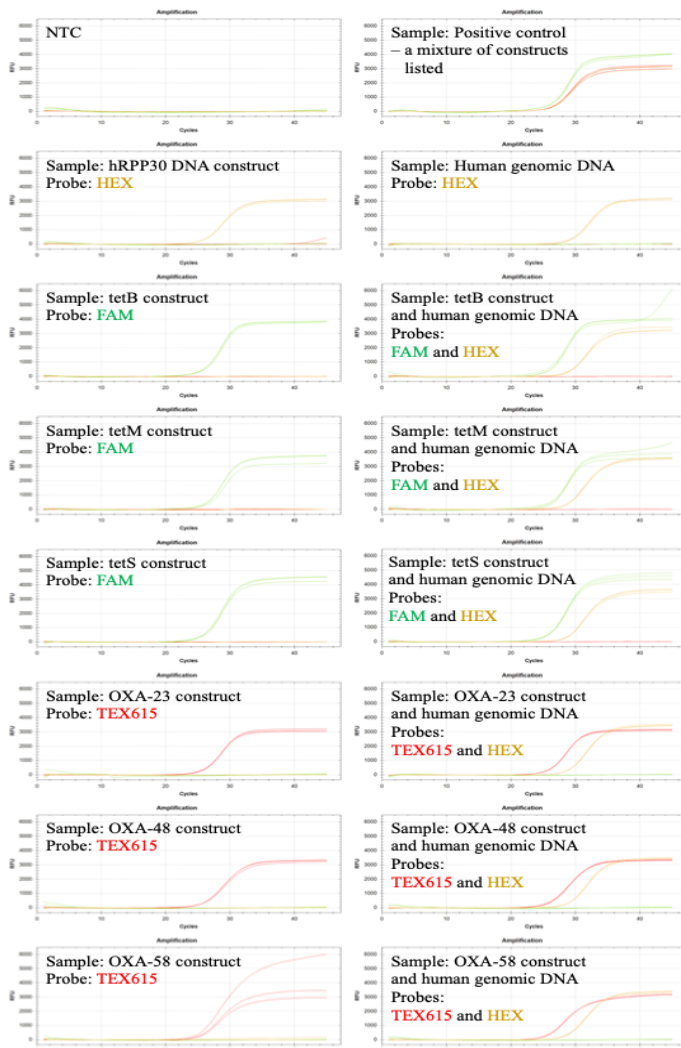


Figure 1: Verification experiments with single and multiple targets. All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

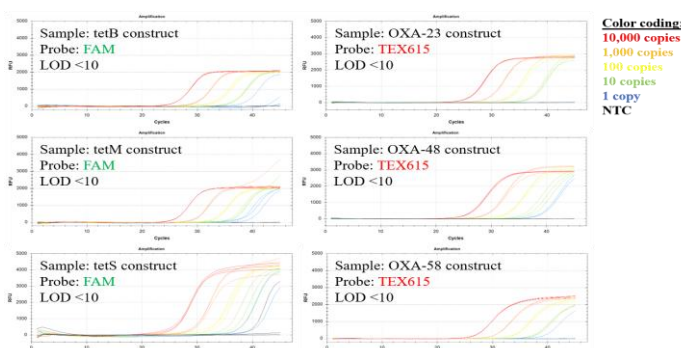


Figure 2: Serial dilution experiments show LOD < 10 molecules for the synthetic DNA construct of each target.

CONTACT US

For assistance, please contact DNA Software using the link:
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