PCRassays.com

ASSAY NAME: AMPC

Quantity: 100 x 20µL PCR reactions

5-plex assay: CMY-2, DHA, FOX, MOX, and human RPP30 DNA

SKU: PNP-AMPC-D-BR-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 (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using PNP-AMPC-D-BR-100 on a BioRad CFX96. 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 AMPC assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 AMR genes and a human extraction control.

Table of Dyes used in this assay:

AMR Gene Target	Dyes	Quencher	Refs.
CMY-2	FAM	BHQ-1	1, 2
RPP30-DNA control	HEX	BHQ-1	3
MOX	TEX615	BHQ-2	4, 5
DHA	Cy5	BHQ-2	6
FOX	Cy5.5	BHQ-2	7

The probes are designed as TaqMan[8] cleavage mechanism, which requires a DNA polymerase with 5'-exonuclease activity. Each AMR gene confers resistance to multiple drugs and in different organisms (see Table below). The primer and probes for each assay detect multiple alleles of each AMR gene (see Table below).

Table of AMR Genes and the Drugs and Organisms where resistance is conferred:

AMR Gene	Drugs	Organisms
CMY-2 group	Cephalosporins, Ceftriaxone, Cefotaxime, Cefixime, Cephamycin, Cefoxitin, Penams, Ampicillin	*Acinetobacter baumannii, Citrobacter freundii, Citrobacter portucalensis, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enterica, Serratia marcescens*
FOX group	Cephalosporin, Cephamycin, Carbapenems	*Aeromonas allosaccharophila, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Providencia rettgeri, Providencia stuartii, Pseudomonas aeruginosa*
MOX group	Cephalosporins, Cefotaxime, Cefepime	*Aeromonas caviae, Escherichia coli, Klebsiella aerogenes, Klebsiella pneumoniae, Proteus mirabilis*
DHA	Cephamycin, Cephalosporin	*Citrobacter freundii, Citrobacter koseri, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis, Salmonella enterica*

ASSAY CONTENTS:

Tube 1: Primer/Probe mix (5X) for CMY-2, DHA, FOX, MOX, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: 5000 copies/µl positive controls of synthetic 500 bp DNA fragments of CMY-2, DHA, FOX, MOX, 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.

ASSAY HANDLING

The AMPC assay is shipped at ambient temperature, 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.

EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended). Use an extraction procedure with an appropriate agent for lysing fungi cells.

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
Molecular biology grade 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.

A PCR protocol was used for verification on a BioRad 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 15 seconds
4	Plate Read
5	Go to Step 2, repeat 44× more

Table of Alleles covered by each PCR assay:

Assay	Alleles Covered by Each PCR Assay
CMY-2 group	CMY-2, CMY-4, CMY-5, CMY-6, CMY-7, CMY-12, CMY-13, CMY-14, CMY-15, CMY-16, CMY-17, CMY-18, CMY-20, CMY-21, CMY-22, CMY-23, CMY-24, CMY-25, CMY-26, CMY-27, CMY-28, CMY-29, CMY-30, CMY-31, CMY-32, CMY-33, CMY-34, CMY-35, CMY-36, CMY-37, CMY-38, CMY-39, CMY-40, CMY-42, CMY-43, CMY-44, CMY-45, CMY-46, CMY-49, CMY-53, CMY-54, CMY-55, CMY-56, CMY-57, CMY-58, CMY-59, CMY-60, CMY-61, CMY-62, CMY-63, CMY-64, CMY-69, CMY-71, CMY-73, CMY-77, CMY-80, CMY-86, CMY-94, CMY-95, CMY-96, CMY-99, CMY-102, CMY-104, CMY-106, CMY-107, CMY-108, CMY-111, CMY-119, CMY-121, CMY-122, CMY-124, CMY-125, CMY-127, CMY-129, CMY-130, CMY-131, CMY-132, CMY-133, CMY-134, CMY-136, CMY-138, CMY-139, CMY-140, CMY-141, CMY-142, CMY-143, CMY-144, CMY-145, CMY-146, CMY-147, CMY-148, CMY-149, CMY-153, CMY-154, CMY-155, CMY-156, CMY-158, CMY-160, CMY-161, CMY-162, CMY-163, CMY-164, CMY-165, CMY-166, CMY-167, CMY-171, CMY-172, CMY-173, CMY-174, CMY-175, CMY-176, CMY-177, CMY-178, CMY-183, CMY-184, CMY-185, CMY-186, BIL, LAT
FOX group	FOX-1, FOX-2, FOX-3, FOX-4, FOX-5, FOX-6, FOX-7, FOX-8, FOX-9, FOX-10, FOX-12, FOX-13, FOX-14, FOX-15, FOX-16, FOX-17, FOX-18, FOX-19, FOX-20, FOX-21
MOX group	CMY-8, CMY-8b, CMY-9, CMY-10, CMY-11, CMY-19, MOX-1, MOX-2, MOX-3, MOX-4, MOX-8, MOX-10, MOX-11, MOX-14, MOX-16, MOX-17, MOX-18, MOX-20, MOX-21, MOX-22, MOX-23
DHA group	DHA-1, DHA-2, DHA-3, DHA-4, DHA-5, DHA-6, DHA-7, DHA-9, DHA-10, DHA-12, DHA-13, DHA-14, DHA-15, DHA-16, DHA-17, DHA-18, DHA-19, DHA-20, DHA-21, DHA-22, DHA-23, DHA-24, DHA-25, DHA-26, DHA-27, DHA-28, DHA-29, DHA-30, DHA-31, DHA-33, MOR-2

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 BioRad CFX-96 with 100 µL wells and 20 µL reaction volume, the average RFU was approximately between 1,000 and 3,000 we used a threshold of 200 for calling positives or "+" in the Table below.

CMY-2 FAM™	MOX TEX615™	DHA Cy5™	FOX Cy5.5™	RPP30 HEX™	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 CMY-2 DNA. The sample may not contain human RPP30 DNA.
+	–	–	–	+	The sample contains CMY-2 DNA and human RPP30 DNA.
–	+	–	–	–	The sample contains MOX DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains MOX DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains DHA DNA. The sample may not contain human RPP30 DNA.
–	–	+	–	+	The sample contains DHA DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains FOX DNA. The sample may not contain human RPP30 DNA.
–	–	–	+	+	The sample contains FOX DNA and human RPP30 DNA.
+	+	+	+	–	The sample contains CMY-2 DNA, MOX DNA, DHA DNA, and FOX DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains CMY-2 DNA, MOX DNA, DHA DNA, FOX DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The AMPC assay verification was carried out as a 5-plex assay, which simultaneously detects DNA from CMY-2, DHA, FOX, MOX, 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 pathogen genomes, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 5-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 Technol., Inc.
[3] HEX™ (Hexachloro-fluorescein), a trademark of Thermo Fisher Scientific.
[4] TEX615™ is a trademark of Thermo Fisher Scientific.
[5] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technol., Inc.
[6] Cy5™, a trademark of GE Healthcare.
[7] Cy5.5™ is a trademark of GE Healthcare.
[8] TaqMan™ is a trademark of Roche Diagnostics, Inc.

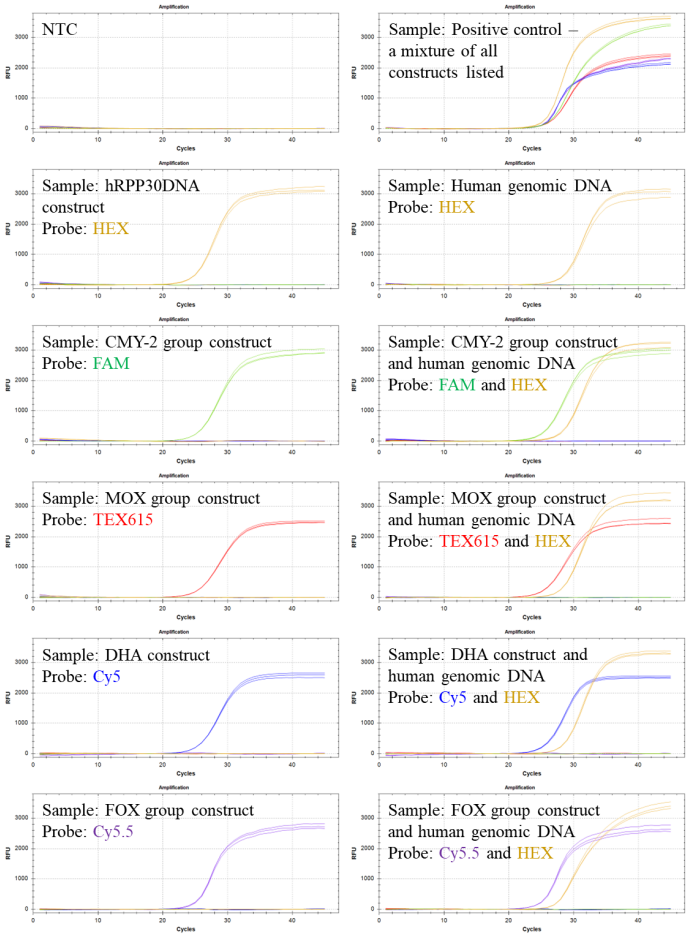

Figure 1: Verification experiments with single and multiple targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

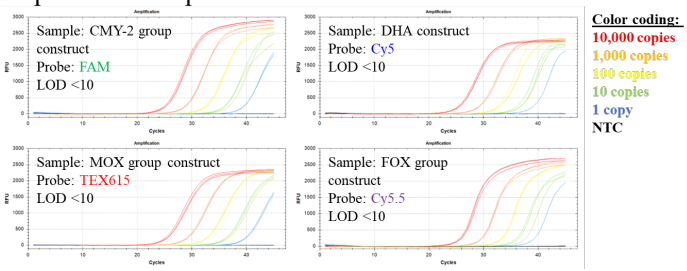

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-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:
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