PCRassays.com

ASSAY NAME: AMPC_QS (AMP-C AMR Panel for QuantStudio)

Quantity: 100 x 20µL PCR reactions
5-plex assay: CMY-2, FOX, MOX, DHA, and human RPP30 DNA

SKU #: PNP-AMPC-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 using PNP-AMPC-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 AMPC_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 AMR genes and a human control.

Table of Dyes used in this assay:

AMR Gene Target	Dyes	Quencher	Refs.
CMY-2	FAM	BHQ-1	1,2
FOX	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4, 5
MOX	TEX615	BHQ-2	6
DHA	Cy5	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: 5X Primer/Probe mix for CMY-2, FOX, MOX, DHA, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for CMY-2, FOX, MOX, DHA, 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 AMPC assay is shipped at ambient temperature, but store 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 PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq 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 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 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, Cq (or use CT if your instrument does not have the capability to compute a Cq). Fluorescence channels with a Cq < 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 reactions, the average RFU was approximately 1,000,000 and we used a threshold of 200,000 for calling positives or "+" in the Table below.

CMY-2 FAM™	FOX HEX ™	hRPP30 TAMRA ™	MOX TEX615 ™	DHA Cy5 ™	Recommended Interpretation
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment
–	–	+	–	–	The sample does not contain pathogen DNA. The sample contains human RPP30 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 FOX DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains FOX 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 CMY-2, FOX, MOX, DHA DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains CMY-2, FOX, MOX, DHA DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The AMPC_QS 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 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the AMR genes, human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1** and indicate that the 5-plex specifically detects the different AMR genes species in the human genomic DNA matrix.

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.

CONTACT US

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

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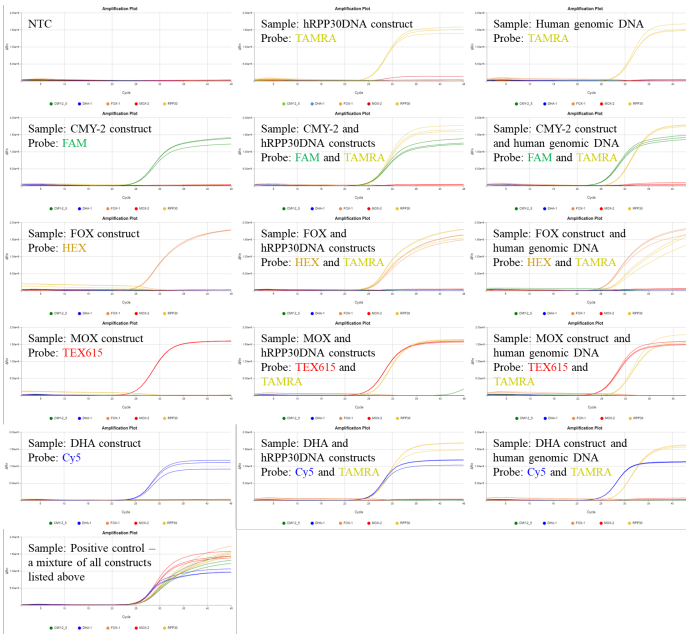

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

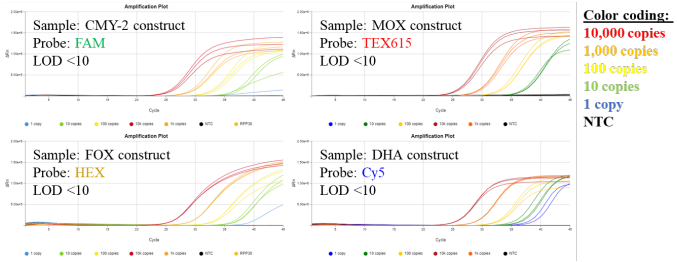

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicate that the 5-plex primers and probes specifically detect and differentiate the AMR genes and are compatible with RPP30_DNA positive control primers. Figure 1 also indicates that human genomic DNA matrix doesn't affect detection of the AMR DNA.

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 Cor.

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

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