PCRassays.com

ASSAY NAME: CARB2_BR

Quantity: 100 x 20µL PCR reactions

5-color assay: NDM-1, VIM, IMP-1, IMP-7, IMP-16, OXA-23, OXA-48, OXA-58, and RPP30 DNA

SKU #: PNP-CARB2-D-BR-100 (BioRad)

(RUO). Research Use Only. Not for use in Diagnostic Procedures.

SCOPE OF THIS DOCUMENT:

The oligonucleotide recipes are optimized for each instrument (QuantStudio, BioRad). The verification data presented in this PIS were performed using PNP-CARB2-D-BR-100 on a BioRad CFX96 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 CARB2_BR assay are provided in Tube 1 as a 5X concentrated working solution that detects 4 AMR genes and a human control. The primer and probes for each assay detect multiple alleles of each AMR gene (see Table below). Each AMR gene confers resistance to multiple drugs and in different organisms (see Table below).

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
NDM-1	FAM	BHQ-1	1, 2
RPP30-DNA control	HEX	BHQ-1	3
VIM	TEX615	BHQ-2	4,5
IMP-1/7/16	Cy5	BHQ-2	6
OXA-23/48/58	Cy5.5	BHQ-2	7

Table of Alleles covered by each PCR assay:

Assay	Alleles Covered by Each PCR Assay
NDM group	NDM-1, NDM-2, NDM-3, NDM-4, NDM-5, NDM-6, NDM-7, NDM-8, NDM-9, NDM-10, NDM-11, NDM-12, NDM-13, NDM-14, NDM-15, NDM-16a, NDM-16b, NDM-17, NDM-18, NDM-19, NDM-20, NDM-21, NDM-22, NDM-23, NDM-24, NDM-25, NDM-26, NDM-27, NDM-28, NDM-29, NDM-30, NDM-31, NDM-33, NDM-34, NDM-35, NDM-36, NDM-37, NDM-38, NDM-39, NDM-40, NDM-41, NDM-42, NDM-43, NDM-44, NDM-45, NDM-46, NDM-47, NDM-48, NDM-49, NDM-50, NDM-51, NDM-52, NDM-53, NDM-54, NDM-55, NDM-56, NDM-57, NDM-58, NDM-60, NDM-61
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
VIM group	VIM-1, VIM-2, VIM-3, VIM-4, VIM-5, VIM-6, VIM-7, VIM-8, VIM-9, VIM-10, VIM-11, VIM-12, VIM-13, VIM-14, VIM-15, VIM-16, VIM-17, VIM-18, VIM-19, VIM-20, VIM-23, VIM-24, VIM-25, VIM-26, VIM-27, VIM-28, VIM-29, VIM-30, VIM-31, VIM-32, VIM-33, VIM-34, VIM-35, VIM-36, VIM-37, VIM-38, VIM-39, VIM-40, VIM-41, VIM-42, VIM-43, VIM-44, VIM-45, VIM-46, VIM-47, VIM-48, VIM-49, VIM-50, VIM-51, VIM-52, VIM-53, VIM-54, VIM-55, VIM-56, VIM-57, VIM-58, VIM-59, VIM-60, VIM-61, VIM-62, VIM-63, VIM-64, VIM-65, VIM-66, VIM-67, VIM-68, VIM-69, VIM-70, VIM-71, VIM-72, VIM-73, VIM-74, VIM-75, VIM-76, VIM-77, VIM-78, VIM-79, VIM-80, VIM-81, VIM-82, VIM-83, VIM-84, VIM-85, VIM-86
IMP group	IMP-1, IMP-3, IMP-4, IMP-5, IMP-6, IMP-7, IMP-10, IMP-11, IMP-15, IMP-16, IMP-21, IMP-22, IMP-25, IMP-26, IMP-28, IMP-29, IMP-30, IMP-34, IMP-38,IMP-40, IMP-41, IMP-42, IMP-43, IMP-44, IMP-51, IMP-52, IMP-55, IMP-58, IMP-59, IMP-60, IMP-61, IMP-62, IMP-66, IMP-68, IMP-70, IMP-73, IMP-74, IMP-76, IMP-77, IMP-78, IMP-79, IMP-80, IMP-81, IMP-85, IMP-88, IMP-89, IMP-93, IMP-94, IMP-97, IMP-98, IMP-102

ASSAY CONTENTS:

Tube 1: 5X Primer/Probe mix for NDM-1, VIM, IMP-1/7/16, OXA-23/48/58, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for NDM-1, VIM, IMP-1/7/16, OXA-23/48/58, 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 CARB2_BR 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.

The probes are designed as TaqMan[8] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

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 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.

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

AMR Gene	Drugs	Organisms
NDM family	Carbapenem	*Acinetobacter baumannii, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Providencia rettgeri, Providencia stuartii, Pseudomonas aeruginosa, Vibrio parahaemolyticus*
OXA-23 group	Penicillin, Carbapenem	*Acinetobacter baumannii, Acinetobacter radioresistens, Klebsiella pneumoniae*
OXA-48 group	Penicillin, Carbapenem	*Citrobacter freundii, Enterobacter cloacae, Enterobacter kobei, Escherichia coli, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Providencia stuartii, Serratia marcescens, Shewanella xiamenensis*
OXA-58 group	Penicillin, Carbapenem	*Acinetobacter baumannii*
VIM family	Carbapenem, Cephalosporin, Cephamycin, Penam, Penem, Imipenem, Meropenem, Aminoglycosides, Amikacin, Gentamicin, Ciprofloxacin, Tobramycin	*Citrobacter freundii, Enterobacter cloacae, Enterobacter hormaechei, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas shirazica, Serratia marcescens, Vibrio alginolyticus*
IMP 1 ,7 ,16 groups	Penam, Carbapenems, Imipenem, Meropenem, Cephamycin, Cephalosporins, Ceftriaxone and Cefazolin, Ertapenem, Ampicillin	*Achromobacter xylosoxidans, Acinetobacter baumannii, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas asiatica, Pseudomonas fluorescens, Pseudomonas putida, Serratia marcescens, Shigella*

A PCR protocol was used for verification on a BioRad CFX96 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 15 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 BioRad CFX96 with 96 well plate with 100 µL wells and 20 µL reaction volume, the average RFU was approximately 2,500 and we used a threshold of 200 for calling positives or "+" in the Table below.

NDM-1 FAM™	RPP30 HEX™	VIM TEX615™	IMP-1/7/16 Cy5™	OXA-23/48/58 Cy5.5™	**Recommended Interpretation**
–	–	–	–	–	The PCR reaction failed. Please repeat the experiment.
–	+	–	–	–	The sample contains human RPP30 DNA. The sample doesn't contain AMR DNA.
+	–	–	–	–	The sample contains NDM-1 DNA. The sample may not contain human RPP30 DNA.
+	+	–	–	–	The sample contains NDM-1 DNA and human RPP30 DNA.
–	–	+	–	–	The sample contains VIM DNA . The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains VIM DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains IMP DNA. The sample may not contain human RPP30 DNA.
–	+	–	+	–	The sample contains IMP DNA and human RPP30 DNA.
–	–	–	–	+	The sample contains OXA DNA. The sample may not contain human RPP30 DNA.
–	+	–	–	+	The sample contains OXA DNA and human RPP30 DNA.
+	–	+	+	+	The sample contains NDM-1, VIM, IMP, and OXA DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains NDM-1, VIM, IMP, and OXA DNA, and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The CARB2_QS assay verification was carried out as a 5-color assay, which simultaneously detects DNA from NDM-1, VIM, IMP-1/7/16, OXA-23/48/58, 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 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.

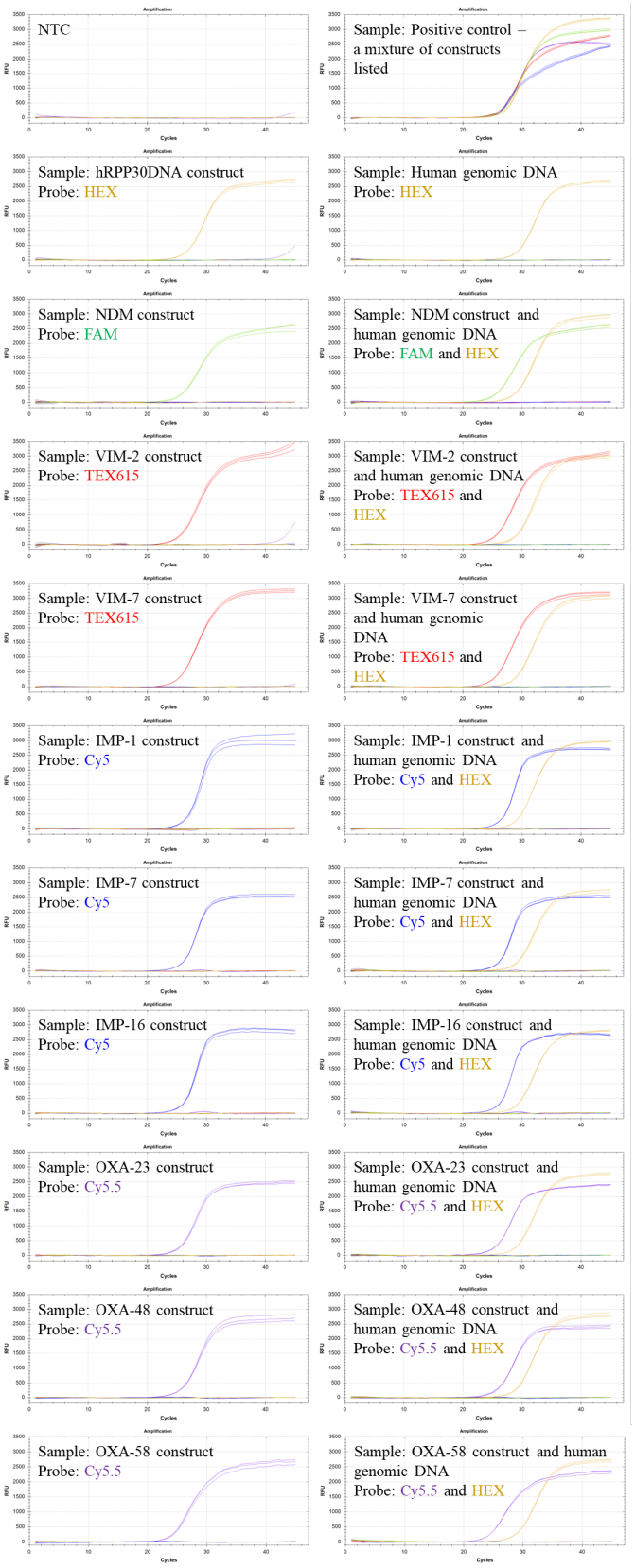

Figure 1: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. Signal from single molecule events show Cq >38 and are considered negative.

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 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] TEX615™ is a trademark of Thermo Fisher Scientific.
[5] BHQ-2™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
[6] Cy5™ is a trademark of GE Healthcare.
[7] Cy5.5™ is a trademark of GE Healthcare.
[8] "TaqMan" is a trademark of Roche Molecular Systems, Inc.

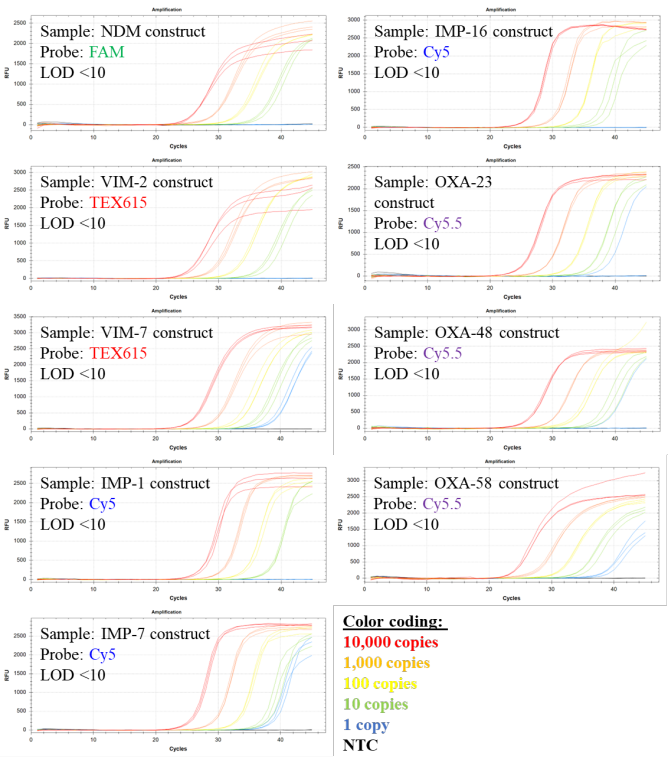

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

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

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