

ASSAY NAME: ESBL_QS (Extended-spectrum beta-lactamases AMR Panel for QuantStudio)

Quantity: 100 x 20µL PCR reactions

5-color assay: CTX-M-1, 2, 9, and 25, KPC, SHV, TEM, and human RPP30 DNA

SKU #: PNP-ESBL-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-ESBL-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 ESBL_QS assay are provided in Tube 1 as a 5X concentrated working solution that detects 6 AMR genes and a human control. The TaqMan⁸ probes require a DNA polymerase with 5'-exonuclease activity.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
CTX-M Groups	FAM	BHQ-1	1,2
KPC	HEX	BHQ-1	3
RPP30-DNA control	TAMRA	BHQ-2	4, 5
SHV	TEX615	BHQ-2	6
TEM	Cy5	BHQ-2	7

Table of AMR Genes, Drugs and Organisms:

AMR Gene	Drugs	Organisms
CTX-M-1 group	Cefotaxime, Ceftriaxone, Ceftazidime, ESBL	<i>Aeromonas hydrophila, Citrobacter freundii, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enterica, Serratia marcescens</i>
		<i>Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Kluyvera ascorbata, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enterica</i>
CTX-M-2 group	Cefotaxime, Ceftriaxone, Ceftazidime, ESBL	<i>Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enterica, Serratia marcescens</i>
CTX-M-9 group	Cefotaxime, Ceftriaxone, Ceftazidime, ESBL	<i>Escherichia coli, Klebsiella pneumoniae, Kluyvera ascorbata, Kluyvera georgiana, Proteus mirabilis, Providencia stuartii</i>
CTX-M-25 group	Cefotaxime, Ceftriaxone, Ceftazidime, ESBL	<i>Acinetobacter baumannii, Citrobacter freundii, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae</i>
SHV family	Carbapenem, Cephalosporin, Penam, ESBL, Monobactams	<i>Acinetobacter baumannii, Acinetobacter haemolyticus, Capnocytophaga ochracea, Citrobacter koseri, Enterobacter cloacae, Enterobacter kobei, Escherichia coli, Haemophilus parainfluenzae, Klebsiella aerogenes, Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella variicola, Morganella morganii, Neisseria gonorrhoeae, Proteus mirabilis, Providencia stuartii, Pseudomonas aeruginosa, Pseudomonas alloputida, Salmonella enterica, Serratia marcescens, Shigella sonnei, Staphylococcus aureus</i>
TEM family	ESBL, Penicillins, Cephalosporins, Monobactams	<i>Acinetobacter baumannii, Citrobacter freundii, Citrobacter koseri, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa</i>
KPC group	ESBL, cephalosporins, monobactams, carbapenems	<i>Acinetobacter baumannii, Citrobacter freundii, Citrobacter koseri, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa</i>

ASSAY CONTENTS:

Tube 1: 5X Primer/Probe mix for CTX-M, KPC, SHV, TEM, and hRPP30DNA.

Tube 2: (Do NOT add to specimen unknowns) Positive control: 5000 copies/µl of synthetic 500 bp DNA fragments for CTX-M, KPC, SHV, TEM, 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 ESBL_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 44x more

Each AMR gene confers resistance to multiple drugs and in different organisms (see Table to the left). The primer and probes for each assay detect multiple alleles of each AMR gene (see Table next page).

Table of Alleles covered by each PCR assay:

Assay	Alleles Covered by Each PCR Assay
CTX-M-1 group	CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12, CTX-M-15, CTX-M-22, CTX-M-23, CTX-M-28, CTX-M-29, CTX-M-30, CTX-M-32, CTX-M-33, CTX-M-34, CTX-M-36, CTX-M-37, CTX-M-42, CTX-M-52, CTX-M-53, CTX-M-54, CTX-M-55, CTX-M-58, CTX-M-60, CTX-M-61, CTX-M-62, CTX-M-66, CTX-M-68, CTX-M-69, CTX-M-70, CTX-M-71, CTX-M-72, CTX-M-79, CTX-M-80, CTX-M-82, CTX-M-88, CTX-M-96, CTX-M-101, CTX-M-103, CTX-M-114, CTX-M-116, CTX-M-117, CTX-M-127, CTX-M-136, CTX-M-138, CTX-M-139, CTX-M-142, CTX-M-143, CTX-M-144, CTX-M-146, CTX-M-150, CTX-M-154, CTX-M-155, CTX-M-156, CTX-M-157, CTX-M-158, CTX-M-162, CTX-M-163, CTX-M-164, CTX-M-166, CTX-M-167, CTX-M-169, CTX-M-170, CTX-M-172, CTX-M-173, CTX-M-175, CTX-M-176, CTX-M-177, CTX-M-178, CTX-M-179, CTX-M-180, CTX-M-181, CTX-M-182, CTX-M-183, CTX-M-184, CTX-M-186, CTX-M-187, CTX-M-188, CTX-M-189, CTX-M-190, CTX-M-193, CTX-M-194, CTX-M-197, CTX-M-202, CTX-M-203, CTX-M-204, CTX-M-206, CTX-M-207, CTX-M-208, CTX-M-209, CTX-M-210, CTX-M-211, CTX-M-212, CTX-M-216, CTX-M-218, CTX-M-220, CTX-M-222, CTX-M-224, CTX-M-225, CTX-M-226, CTX-M-227, CTX-M-228, CTX-M-230, CTX-M-231, CTX-M-232, CTX-M-236, CTX-M-237, CTX-M-238, CTX-M-244, CTX-M-245, CTX-M-246, CTX-M-251, CTX-M-254, CTX-M-256, CTX-M-257, CTX-M-258, CTX-M-259, CTX-M-260, CTX-M-261, CTX-M-262, CTX-M-263, CTX-M-264, CTX-M-265, CTX-M-266, CTX-M-268
CTX-M-2 group	CTX-M-2, CTX-M-4, CTX-M-5, CTX-M-6, CTX-M-7, CTX-M-20, CTX-M-31, CTX-M-35, CTX-M-43, CTX-M-44, CTX-M-56, CTX-M-59, CTX-M-76, CTX-M-77, CTX-M-92, CTX-M-95, CTX-M-97, CTX-M-115, CTX-M-124, CTX-M-131, CTX-M-141, CTX-M-165, CTX-M-171, CTX-M-200, CTX-M-229, CTX-M-253
CTX-M-9 group	CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-24, CTX-M-27, CTX-M-38, CTX-M-46, CTX-M-47, CTX-M-48, CTX-M-49, CTX-M-50, CTX-M-51, CTX-M-65, CTX-M-67, CTX-M-73, CTX-M-81, CTX-M-83, CTX-M-84, CTX-M-85, CTX-M-86, CTX-M-87, CTX-M-90, CTX-M-93, CTX-M-98, CTX-M-99, CTX-M-102, CTX-M-104, CTX-M-105, CTX-M-110, CTX-M-111, CTX-M-112, CTX-M-113, CTX-M-121, CTX-M-122, CTX-M-125, CTX-M-126, CTX-M-129, CTX-M-130, CTX-M-134, CTX-M-137, CTX-M-140, CTX-M-147, CTX-M-148, CTX-M-159, CTX-M-161, CTX-M-168, CTX-M-174, CTX-M-191, CTX-M-192, CTX-M-195, CTX-M-196, CTX-M-198, CTX-M-201, CTX-M-213, CTX-M-214, CTX-M-215, CTX-M-219, CTX-M-221, CTX-M-223, CTX-M-233, CTX-M-235, CTX-M-239, CTX-M-240, CTX-M-241, CTX-M-242, CTX-M-243, CTX-M-252, CTX-M-255, CTX-M-267, CTX-M-269
CTX-M-25 group	CTX-M-25, CTX-M-26, CTX-M-39, CTX-M-41, CTX-M-78, CTX-M-89, CTX-M-91, CTX-M-94, CTX-M-100, CTX-M-152, CTX-M-160, CTX-M-185, CTX-M-205, CTX-M-217
SHV group	SHV-1, SHV-1b-b, SHV-2, SHV-2a, SHV-3, SHV-4, SHV-5, SHV-7, SHV-8, SHV-9, SHV-11, SHV-12, SHV-13, SHV-14, SHV-15, SHV-16, SHV-18, SHV-24, SHV-25, SHV-26, SHV-27, SHV-28, SHV-29, SHV-30, SHV-31, SHV-32, SHV-33, SHV-34, SHV-35, SHV-36, SHV-37, SHV-38, SHV-40, SHV-41, SHV-42, SHV-43, SHV-44, SHV-45, SHV-46, SHV-48, SHV-49, SHV-50, SHV-51, SHV-52, SHV-55, SHV-56, SHV-57, SHV-59, SHV-60, SHV-61, SHV-62, SHV-63, SHV-64, SHV-65, SHV-66, SHV-67, SHV-69, SHV-70, SHV-71, SHV-72, SHV-73, SHV-74, SHV-75, SHV-76, SHV-77, SHV-78, SHV-79, SHV-80, SHV-81, SHV-82, SHV-85, SHV-86, SHV-89, SHV-92, SHV-93, SHV-94, SHV-95, SHV-96, SHV-97, SHV-98, SHV-99, SHV-100, SHV-101, SHV-102, SHV-103, SHV-104, SHV-105, SHV-106, SHV-107, SHV-108, SHV-109, SHV-110, SHV-111, SHV-112, SHV-115, SHV-116, SHV-119, SHV-120, SHV-121, SHV-122b, SHV-128, SHV-129, SHV-132, SHV-133, SHV-134, SHV-135, SHV-137, SHV-141, SHV-142, SHV-143, SHV-144, SHV-145, SHV-146, SHV-147, SHV-148, SHV-149, SHV-150, SHV-151, SHV-152, SHV-153, SHV-154, SHV-155, SHV-156, SHV-157, SHV-158, SHV-159, SHV-160, SHV-161, SHV-162, SHV-163, SHV-164, SHV-165, SHV-168, SHV-171, SHV-172, SHV-173, SHV-178, SHV-179, SHV-180, SHV-182, SHV-183, SHV-185, SHV-186, SHV-187, SHV-188, SHV-189, SHV-190, SHV-191, SHV-193, SHV-194, SHV-195, SHV-196, SHV-197, SHV-198, SHV-199, SHV-200, SHV-201, SHV-202, SHV-203, SHV-204, SHV-205, SHV-206, SHV-207, SHV-208, SHV-209, SHV-210, SHV-211, SHV-212, SHV-213, SHV-214, SHV-215, SHV-216, SHV-217, SHV-218, SHV-219, SHV-220, SHV-221, SHV-222, SHV-223, SHV-224, SHV-225, SHV-226, SHV-227, SHV-228, SHV-229, SHV-230, SHV-231, SHV-232
TEM group	TEM-1, TEM-2, TEM-3, TEM-4, TEM-5, TEM-6, TEM-7, TEM-8, TEM-9, TEM-10, TEM-10, TEM-11, TEM-12, TEM-15, TEM-16, TEM-17, TEM-19, TEM-20, TEM-21, TEM-22, TEM-24, TEM-26, TEM-28, TEM-29, TEM-30, TEM-31, TEM-32, TEM-33, TEM-34, TEM-35, TEM-36, TEM-37, TEM-39, TEM-40, TEM-43, TEM-45, TEM-47, TEM-48, TEM-49, TEM-52, TEM-53, TEM-54, TEM-55, TEM-57, TEM-60, TEM-61, TEM-63, TEM-67, TEM-68, TEM-70, TEM-71, TEM-72, TEM-76, TEM-77, TEM-78, TEM-79, TEM-80, TEM-81, TEM-82, TEM-83, TEM-84, TEM-85, TEM-86, TEM-87, TEM-88, TEM-90, TEM-91, TEM-92, TEM-93, TEM-94, TEM-95, TEM-96, TEM-97, TEM-98, TEM-99, TEM-101, TEM-102, TEM-103, TEM-104, TEM-105, TEM-106, TEM-107, TEM-108, TEM-109, TEM-110, TEM-111, TEM-112, TEM-113, TEM-114, TEM-115, TEM-116, TEM-120, TEM-121, TEM-122, TEM-123, TEM-124, TEM-125, TEM-126, TEM-127, TEM-128, TEM-129, TEM-130, TEM-131, TEM-132, TEM-133, TEM-134, TEM-135, TEM-136, TEM-137, TEM-138, TEM-139, TEM-141, TEM-142, TEM-143, TEM-144, TEM-145, TEM-146, TEM-147, TEM-148, TEM-149, TEM-150, TEM-151, TEM-152, TEM-153, TEM-154, TEM-155, TEM-156, TEM-157, TEM-158, TEM-159, TEM-160, TEM-162, TEM-163, TEM-164, TEM-166, TEM-167, TEM-168, TEM-169, TEM-171, TEM-176, TEM-177, TEM-178, TEM-181, TEM-182, TEM-183, TEM-184, TEM-185, TEM-186, TEM-187, TEM-188, TEM-189, TEM-190, TEM-191, TEM-193, TEM-194, TEM-195, TEM-196, TEM-197, TEM-198, TEM-201, TEM-205, TEM-206, TEM-207, TEM-208, TEM-209, TEM-210, TEM-211, TEM-212, TEM-213, TEM-214, TEM-215, TEM-216, TEM-216, TEM-217, TEM-219, TEM-220, TEM-224, TEM-225, TEM-226, TEM-227, TEM-228, TEM-229, TEM-230, TEM-231, TEM-232
KPC group	KPC-2, KPC-3, KPC-4, KPC-5, KPC-6, KPC-7, KPC-8, KPC-10, KPC-11, KPC-12, KPC-13, KPC-14, KPC-15, KPC-16, KPC-17, KPC-18, KPC-19, KPC-21, KPC-22, KPC-23, KPC-24, KPC-25, KPC-26, KPC-27, KPC-28, KPC-29, KPC-30, KPC-31, KPC-32, KPC-33, KPC-34, KPC-35, KPC-36, KPC-37, KPC-38, KPC-39, KPC-40, KPC-41, KPC-42, KPC-43, KPC-44, KPC-45, KPC-46, KPC-47, KPC-48, KPC-49, KPC-50, KPC-51, KPC-52, KPC-53, KPC-54, KPC-55, KPC-56, KPC-57, KPC-58, KPC-59, KPC-60, KPC-61, KPC-62, KPC-63, KPC-64, KPC-65, KPC-66, KPC-67, KPC-68, KPC-69, KPC-70, KPC-71, KPC-72, KPC-73, KPC-74, KPC-75, KPC-76, KPC-77, KPC-78, KPC-79, KPC-80, KPC-81, KPC-82, KPC-83, KPC-84, KPC-85, KPC-86, KPC-87, KPC-88, KPC-89, KPC-90, KPC-91, KPC-92, KPC-93, KPC-94, KPC-95, KPC-96, KPC-97, KPC-98, KPC-99, KPC-100, KPC-101, KPC-102, KPC-103, KPC-104, KPC-105, KPC-106, KPC-107, KPC-108, KPC-109, KPC-110, KPC-111, KPC-112, KPC-113, KPC-114, KPC-115, KPC-116, KPC-117, KPC-118, KPC-119, KPC-120, KPC-121, KPC-122, KPC-123, KPC-124, KPC-125, KPC-126, KPC-127, KPC-128, KPC-129, KPC-130, KPC-131, KPC-132, KPC-133, KPC-134, KPC-135, KPC-136, KPC-137, KPC-138, KPC-139, KPC-140, KPC-141, KPC-142, KPC-143, KPC-144, KPC-145, KPC-146, KPC-147, KPC-148, KPC-151, KPC-153, KPC-154, KPC-155, KPC-156, KPC-157, KPC-158, KPC-159, KPC-160, KPC-161, KPC-162, KPC-163, KPC-164, KPC-165, KPC-166, KPC-167, KPC-168, KPC-170, KPC-176, KPC-178, KPC-179, KPC-180, KPC-181, KPC-182, KPC-183, KPC-184, KPC-185, KPC-186, KPC-187, KPC-189, KPC-190, KPC-191, KPC-192, KPC-193, KPC-194, KPC-195, KPC-196, KPC-197, KPC-201

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 QuantStudio™ 7 Flex with 96 well plate with 100 μ L wells and 20 μ L reaction volume, the average RFU was approximately 200,000 and we used a threshold of 20,000 for calling positives or “+” in the Table below.

CTX-M FAM™	KPC HEX™	hRP P30 TA MR AT™	SHV TEX 615™ M	TEM Cys™	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 CTX-M DNA. The sample may not contain human RPP30 DNA.
+	+	–	–	–	The sample contains KPC DNA. The sample may not contain human RPP30 DNA.
–	+	+	–	–	The sample contains KPC DNA and human RPP30 DNA.
–	–	–	+	–	The sample contains SHV DNA. The sample may not contain human RPP30 DNA.
–	–	+	+	–	The sample contains SHV DNA and human RPP30 DNA.
–	–	–	–	+	The sample contains TEM DNA. The sample may not contain human RPP30 DNA.
+	+	–	+	+	The sample contains CTX-M, KPC, SHV, TEM DNA. The sample may not contain human RPP30 DNA.
+	+	+	+	+	The sample contains CTX-M, KPC, SHV, TEM DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The ESBL_QS assay verification was carried out as a 5-color assay, which simultaneously detects DNA from CTX-M, KPC, SHV, TEM, 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 **Figures 1A and 1B** 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.

Conclusion: The data in **Figure 1** indicate that the primers and probes of the ESBL assay 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.

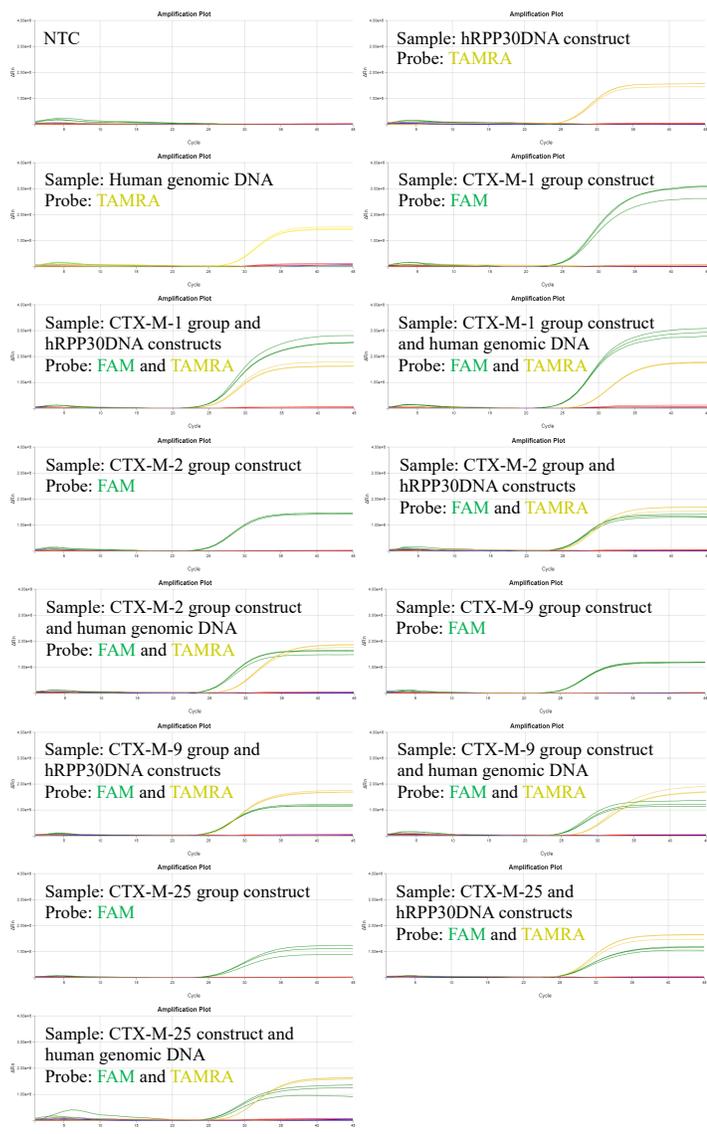


Figure 1A: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

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.
- 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.

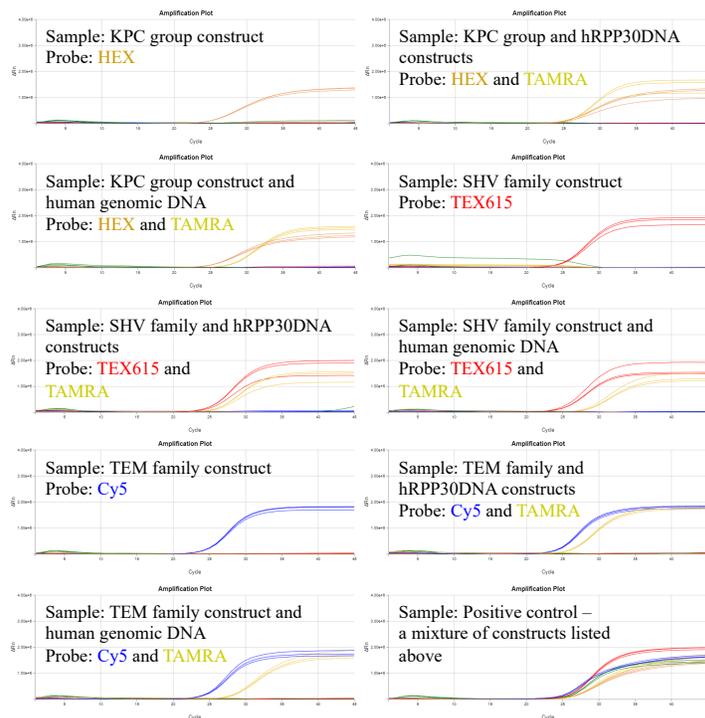


Figure 1B: Verification experiments with single targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

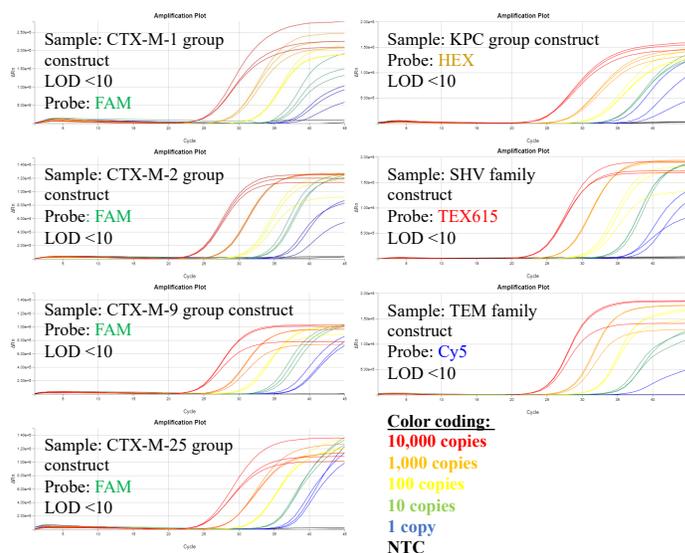


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

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

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