

ASSAY NAME: *Enterobacter cloacae*

Quantity: 100 x 20µL PCR reactions

2-plex assay: *Enterobacter cloacae* and human RPP30 DNA

SKU#:

PNP-ECLO-D-QS (QuantStudio with control assay)

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

SCOPE OF THIS DOCUMENT

The oligonucleotide recipes are optimized for each instrument (BioRad, QuantStudio, MIC). The verification data presented in this document were performed using PNP-WND7-D-QS-100 on a QuantStudio 7 Flex. 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 ECLO assay are provided in Tube 1 as a 20X concentrated working solution that detects *Enterobacter cloacae* and a human control.

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
<i>Enterobacter cloacae</i>	Cy5	BHQ-2	1, 2
RPP30-DNA control	TAMRA	BHQ-2	2, 3

The probes are designed as TaqMan⁴ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

KIT HANDLING AND CONTAMINATION

The ECLO bundle is shipped on ice, 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.

Any contamination should be avoided by using appropriate personal protective equipment (PPE), powder free gloves, aerosol barrier pipette tips, and a clean hood.

Assay contents:

Tube 1: 20X Primer/Probe mix for *Enterobacter cloacae* and hRPP30DNA

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments of *Enterobacter cloacae* and hRPP30DNA.

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.



EXPERIMENTAL

Perform nucleic acid extraction/purification (recommended).

Set up your PCR reaction (20 µL) as follows on ice:

Component	Volume (µL)
InhibiTaq Standard qPCR enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Molecular biology grade water (Not included)	7

Notes: To improve assay sensitivity, up to 9 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. Molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.

A PCR protocol was used for verification on a QuantStudio 7 Flex, 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 44xmore

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, C_q. (C_q is preferred over C_t). Each fluorescence channel with a C_q < 38 cycles and final RFU > “threshold” is considered “positive” or “+” in the Table below. The “threshold” is 2.0 on the BMS MIC, 200 on BioRad instruments and 200,000 on QuantStudio 5, 6, 7, 12K instruments.

<i>Enterobacter cloacae</i> Cys TM	hRPP30 TAMRA TM	Recommended Interpretation
—	—	The PCR reaction failed. Please repeat the experiment.
—	+	The sample does not contain bacterial DNA of interest. The sample contains human RPP30 DNA.
+	—	The sample contains <i>Enterobacter cloacae</i> DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains <i>Enterobacter cloacae</i> DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The *Enterobacter cloacae* primers and probes were previously verified as part of the study of a 5-plex assay (SKU#: PNP-WND7-D-QS-100), which simultaneously detects DNA from *Candida* genus, *S. epidermidis*, *P. aeruginosa*, *E. cloacae*, and human RPP30 DNA, which serves as a positive extraction-control assay. Data from the *Enterobacter cloacae* and control channel are presented below to show the performance expected for the ECLO assay.

Experiments were performed in triplicate using the experimental procedure given above, but with different samples added to each reaction. The samples for verification experiments contained 1×10⁴ copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from the target genome and the human RPP30 DNA gene, and human genomic DNA. The results of these experiments are shown in **Figure 1**.

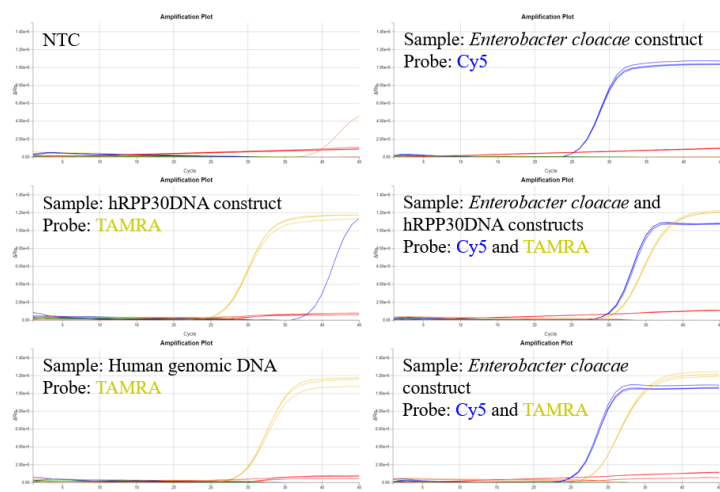


Figure 1: Verification experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

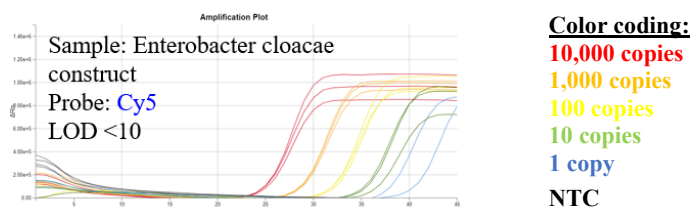


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicates that the *Enterobacter cloacae* primers and probe are compatible with DNAS RPP30 DNA positive control primers and probe in the human genomic DNA matrix.

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

CONTACT US

For assistance, please contact DNA Software using the link:
<https://dnasoft.jira.com/service/customer/portals>

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NOTES

- ¹ CysTM is a trademark of GE Healthcare
- ² BHQ-2TM (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.
- ³ TAMRA (Carboxyltetramethylrhodamine) is a trademark of Applera Corp.
- ⁴ “TaqMan” is a trademark of Roche Molecular Systems, Inc.