

ASSAY NAME: *Cyclospora cayetanensis* (CCAY)
(for BioRad CFX96)

Quantity: 100 x 20µL PCR reactions

2-plex assay: *Cyclospora cayetanensis* and human RPP30 DNA

SKU#:
BUN-CCAY-D-BR-100

(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 BUN-CCAY-D-BR-100 on a BioRad CFX96. Note that this kit is NOT compatible with BioRad CFX-384 instruments. 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 CCAY assay are provided in Tube 1 as a 5X concentrated working solution that detects *Cyclospora cayetanensis* and a human control.

Table of Dyes used in this kit:

Pathogen/Target	Dyes	Quencher	Refs.
<i>Cyclospora cayetanensis</i>	Cy5.5	BHQ-2	1, 2
RPP30-DNA control	HEX	BHQ-1	3, 4

The probes are designed as TaqMan⁵ cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity.

KIT HANDLING AND CONTAMINATION

The CCAY 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: 5X Primer/Probe mix for *Cyclospora cayetanensis* and hRPP30DNA

Tube 2: (Do NOT add to specimen unknowns) Positive control: Synthetic 500 bp DNA fragments of *Cyclospora cayetanensis* 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 (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. For 10 µL reactions, divide all of the amounts above by a factor of 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 44xmore

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). PCR reactions are considered “positive” or “+” in the table below if the RFU > “threshold” and the C_q <38 cycles. “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 Bio-Rad CFX-96 with 100 μ L wells and 20 μ L reaction volume, the average RFU was approximately 3,000, we used a threshold of 300 for calling positives or “+” in the Table below.

<i>Cyclospora cayetanensis</i> Cy5.5™	hRPP30 HEX™	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>Cyclospora cayetanensis</i> DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains <i>Cyclospora cayetanensis</i> DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The *Cyclospora cayetanensis* primers and probes were previously verified as part of the study of a 5-plex assay (SKU#: BUN-GIPARA-D-BR-100), which simultaneously detects DNA *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia intestinalis*, and human RPP30 DNA, which serves as a positive extraction-control assay. Data from the *Cyclospora cayetanensis* and control channel are presented below to show the performance expected for the CCAY 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 1×10^4 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**.

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.

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

NOTES

¹ Cy5.5™ is a trademark of GE Healthcare.

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

³ HEX™ (Hexachloro-fluorescein) is a trademark of Applera Corp.

⁴ BHQ-1™ (Black Hole Quencher) is a trademark of Biosearch Technologies, Inc.

⁵ “TaqMan” is a trademark of Roche Molecular Systems, Inc.

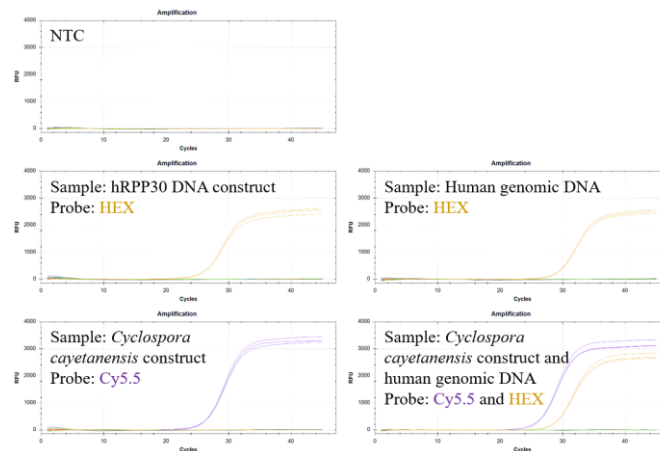


Figure 1: Verification experiments with single or double target(s) (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

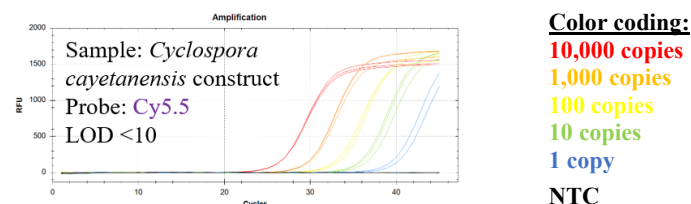


Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

CONTACT US

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

Address: Michigan Life Science and Innovation Center,
46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080