PCRassays.com

ASSAY NAME: PJIR-D-QS

Quantity: 100 x 20µL PCR reactions

2-plex assay: *Pneumocystis jirovecii* and human RPP30 DNA

SKU: BUN-PJIR-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 (BioRad, QuantStudio, MIC). The verification data presented in this PIS were performed using BUN-PJIR-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 need to use a different qPCR instrument.

CONTENTS

The primers and probes in the PJIR assay are provided in Tube 1 as a 20X concentrated working solution that detects *P. jirovecii* and a human extraction control.

Table of Dyes used in this assay:

Pathogen/Target	Dyes	Quencher	Refs.
P. jirovecii	FAM	BHQ-1	1,2
RPP30-DNA control	TAMRA	BHQ-2	3,4

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

ASSAY HANDLING AND CONTAMINATION

The PJIR assay 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.

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: Primer/Probe mix (20X) for *P. jirovecii*, and hRPP30DNA.

Tube 2: (**Do NOT add to specimen unknowns**) Positive control: Synthetic 500 bp DNA fragments of *P. jirovecii*, and human RPP30DNA.

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

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

Component	Volume (µL)
InhibiTaq qPCR enzyme mastermix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water	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 (NOT included) should be used to prepare the PCR reactions.

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

RESULT INTERPRETATION

After running the qPCR reaction, perform a regression analysis on the data to determine the quantification cycle, Cq. (Cq is preferred over Ct). Each fluorescence channel with a Cq < 38 cycles and final RFU > "threshold" is considered "positive" or "+" in the Table below. The "threshold" is 200,000 on QuantStudio instruments.

P. jirovecii FAM™	RPP30 TAMRA™	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample contains human RPP30 DNA. The sample doesn't contain bacterial DNA.
+	–	The sample contains *P. jirovecii* DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains *P. jirovecii* DNA and human RPP30 DNA.

VERIFICATION EXPERIMENTS

The PJIR primers and probes were previously verified as part of the study of a 5-plex assay (SKU#: PNP-FUN4-D-QS-100), which simultaneously detects DNA from *Pneumocystis jirovecii*, Sarocladium spp., *Scopulariopsis brevicaulis*, *Trichophyton spp.,* and human RPP30 DNA, which serves as a positive extraction-control assay. Data from the *P. jirovecii* and control channel are presented below to show the performance expected for the PJIR kit.

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 *P. jirovecii* genome, human RPP30 DNA gene, and human genomic DNA. **Figure 1** shows the results of these experiments, which indicate that the 2-plex specifically detects *P. jirovecii*.

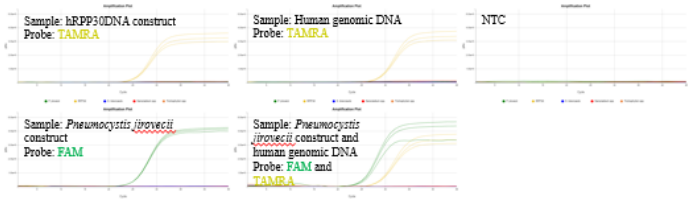

Figure 1: Verification experiments with single and double targets (given in text boxes for each panel). All sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific.

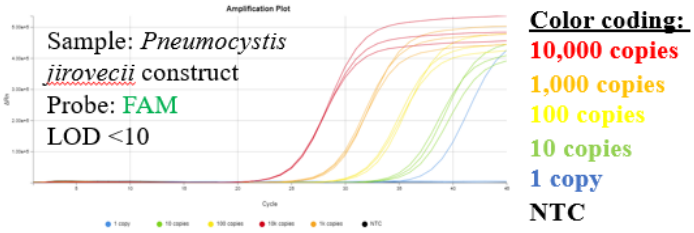

Figure 2: Serial dilution experiments show LOD <10 molecules for the synthetic DNA construct of each target.

Conclusion: The data in **Figure 1** indicates that the *P. jirovecii* 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 *P. jirovecii*.

CONTACT US

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

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46701 Commerce Center Dr, Plymouth, MI 48170

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NOTES

[1] FAM™ (Carboxyfluorescein) is a trademark of Life Technologies Corporation.

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

[3] TAMRA™ (Carboxyltetramethylrhodamine) is a trademark of Applera Cor

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

[5] TaqMan™ is a trademark of Roche Diagnostics, Inc.