Kit: CMV-RPP30DNA
Quantity: 100 x 20µL PCR reactions
Duplexed assay: Cytomegalovirus and human genomic RPP30 DNA
Gene: UL128 (complement)
SKU: PNP-CMV-D-100

Kit contents:
Tube 1: 20X Primer/Probe mix for Cytomegalovirus and hRPP30
Tube 2: 5000 copies/µl Positive controls of synthetic DNA for both Cytomegalovirus and hRPP30.

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

CONTENTS

The CMV-RPP30DNA kit contains a mixture of primers/probe targeting the gene of UL128 in the Cytomegalovirus genome (CMV). The primers and probes in Tube 1 are provided as a 20X concentrated working solution. The fluorophore of the Cytomegalovirus probe is FAM™ and the quencher is BHQ-1™.[1,2] The same mix also contains primers/probe targeting human RPP30DNA Intron I (20X concentrated) as a PCR positive control assay for human samples. The fluorophore of the RPP30DNA probe is HEX™ and the quencher is BHQ-1™.[3] The probes are designed as TaqMan[4] cleavage mechanism and thus the reaction requires a DNA polymerase with 5'-exonuclease activity (we recommend ORA™ qPCR Probe Mix, from HighQu GmbH, Cat number: QPP0101).

Tube 2 contains a mixture of synthetic 500 bp DNA constructs containing the amplicon regions of Cytomegalovirus and hRPP30 is provided as a positive extraction control. The concentration of each DNA construct is approximately 5,000 copies/µL. The Control DNA constructs are for validation purposes only and **Tube 2 should NOT be added to wells for specimen unknowns**.

Note: molecular biology grade water should be used to prepare the PCR reactions, which is NOT included in this kit.

KIT HANDLING AND CONTAMINATION

The CMV-RPP30DNA kit is shipped at ambient temperature, and should be stored at -30 to -15°C. The kit should be kept on ice once thawed.

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

EXPERIMENTAL

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

Component	Volume (µL)
Enzyme: ORA™ qPCR Probe Mix (2X)	10
Primer/Probe mix (20X)	1
Sample	2
Water	7

Note: The composition of this reaction is calculated based on the user manual of ORA™ qPCR Probe Mix, from HighQu GmbH. In a reaction with the double positive control, 2 µL of the solution from Tube 2 should be added.

A PCR protocol was used in-house for pre-validation on a Bio-Rad 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 44xmore

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 >200 is considered "positive" or "+" in the Table below.

Cytomegalovirus (FAM™)	RPP30 (HEX™)	Recommended Interpretation
–	–	The PCR reaction failed. Please repeat the experiment.
–	+	The sample doesn't contain Cytomegalovirus DNA.
+	–	The sample contains Cytomegalovirus DNA. The sample may not contain human RPP30 DNA.
+	+	The sample contains Cytomegalovirus DNA and human RPP30 DNA.

PRE-VALIDATION EXPERIMENTS

The CMV-RPP30DNA kit validation was carried out as a duplexed assay, which simultaneously detects DNA from Cytomegalovirus and DNA from the human RPP30DNA gene, 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 validation experiments contained 1×10^4 copies/reaction of synthetic 500 bp synthetic DNA constructs (from Twist Biosciences) harboring the regions of interest from Cytomegalovirus genome and the RPP30DNA gene. The results of these experiments are shown in **Figure 1** below:

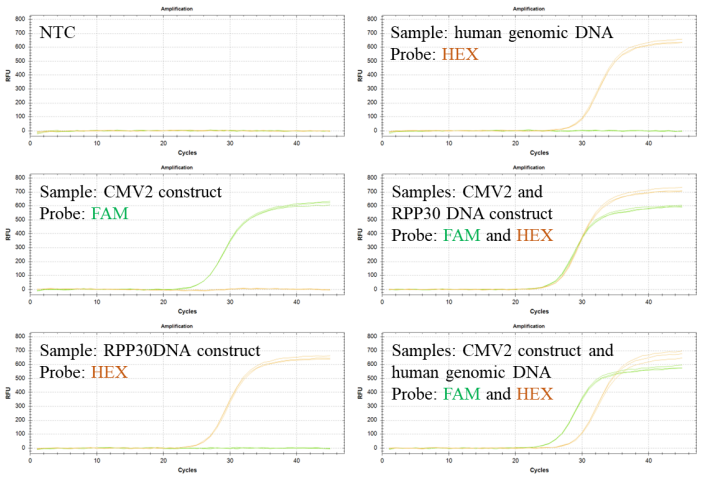

Figure 1: Validation experiments with single or double target(s) (given in text boxes for each panel). Both sets of probes and primers are present in every reaction, but positive signal is only observed when the target(s) is present, indicating that the amplification is specific. The **FAM** probe detects Cytomegalovirus construct DNA. The **HEX** probe detects RPP30DNA construct DNA.

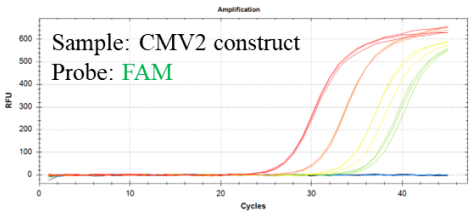

Figure 2: Serial dilution experiments show LOD <10 molecules for Cytomegalovirus 500 bp synthetic DNA construct.

Conclusion: The data in **Figure 1** indicate that the Cytomegalovirus primers and probe are compatible with DNAS RPP30DNA positive control primers and probe in a 2-plex application to detect Cytomegalovirus in the matrix of human sample extract.

Limit of detection (LOD) was estimated by performing serial dilution experiments in triplicate (**Figure 2**). For dilution series only Cytomegalovirus construct was added. The results show a limit of detection (LOD) <10 copies/reaction.

CONTACT US

For assistance, please contact DNA Software, Inc. (the parent company of PCRassays.com) using the link:
https://www.dnasoftware.com/contact/

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46701 Commerce Center Dr, Plymouth, MI 48170

Phone: (734) 222-9080

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), a trademark of Applera Corp.

[4] "TaqMan" is a trademark of Roche Molecular Systems, Inc.