

## Quantiplus® PARVO Real Time Quantitative PCR Kit



QT-PRV-25 : 25 rxns  
 QT-PRV-50 : 50 rxns

**RUO**  
 PI/QTPRV-00

### Introduction & Product Description

Parvo viruses are linear, non-segmented, single-stranded DNA viruses also referred as **B19 virus, parvovirus B19** or sometimes **erythrovirus B19**, was the first (and until 2005 the only) known human virus in the family *Parvoviridae*, genus *Erythroparvovirus*. The name is derived from Latin, parvum meaning small, reflecting the fact that B19 ranks among the smallest DNA viruses. Quantiplus® PARVO Real Time Quantitative PCR Kit is a *Research use* kit for Quantitation of PARVO VIRUS in Blood- EDTA and Urine. The kit contains the necessary reagents for performing quantitation by Real time PCR.

### Kit components

Color Coding (Caps)	Contents	Description	25 rxns (QT-PRV-25)	50 rxns (QT-PRV-50)
Amber	PARVO Ready Mix	PARVO, internal control probes, primers with amplification Mix	1 x 375µL	1 x 750µL
Natural	Huwel IC-B Mix	Internal Control	1 x 300 µL	1 x 600 µL
Pink	Huwel PVQS1	2 X 10 <sup>5</sup> copies/µL	1 x 50 µL	1 x 100 µL
Pink	Huwel PVQS2	2 X 10 <sup>4</sup> copies/µL	1 x 50 µL	1 x 100 µL
Pink	Huwel PVQS3	2 X 10 <sup>3</sup> copies/µL	1 x 50 µL	1 x 100 µL
Pink	Huwel PVQS4	2 X 10 <sup>2</sup> copies/µL	1 x 50 µL	1 x 100 µL
White	Huwel PW	Purified water	1 x 500 µL	1 x 500 µL

### Sample Type

Plasma (K<sub>2</sub>EDTA-Blood) and Urine. *Heparinized Blood must not be used as they inhibit the PCR reaction*

### Assay Procedure

#### DNA Extraction

Sl. No.	Name of the DNA Isolation Kit	Recommended Sample volume (To be taken for DNA Extraction)	Recommended Final Elution volume
1.	Huwel Nucleic Acid Extraction Kit (HL-NAX-100/250)	200 µL	50 µL
2.	Roche High Pure Viral Nucleic Acid kit (Cat. No. 11858874001)	200 µL	50 µL
3.	QIAamp DNA Blood Mini Kit (Cat. No. 51104)	200 µL	50 µL

## Quick Reference Guide qPCR Protocol

Components	Volume per reaction (µL) (For final vol. of 26 µL)
PARVO Ready Mix	15.0
Huwel IC-B Mix (if not added at extraction step)	1.0
Extracted DNA/ Huwel PVQS1-Huwel PVQS4 /Huwel PW	10.0

\*Note: Set the reaction volume as 25 µL (the final volume is 26.0 µL but selecting 25 µL doesn't make any difference to the final result/sensitivity).

### Cycling conditions

Steps	No. of cycles	Temperature (°C)	Time
1 (Initial denaturation)	1	95	15 min.
2 (PCR cycling)	40	95	15 sec.
		60*	60 sec.
* Plate Read/Data Acquisition in <b>FAM</b> and <b>Yakima Yellow / VIC</b> channel			

- Set the reaction volume as 25 µL (the final volume is 26 µL but selecting 25 µL doesn't make any difference to the final result/sensitivity).

### Data Analysis

#### Setting the threshold for the qPCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to  $R_n$ /Cycle amplification plot.

### Result and Interpretation

S.No	FAM (PARVO)	Yakima Yellow (IC)	Fluorophore		Conclusion
			Interpretation		
1	√	√	PARVO DNA within quantitation range	↓	Proceed for further Analysis
2	√	-			
3	-	√	PARVO DNA below quantitation limit		Dilute the DNA sample (1:10) and repeat the Assay
4	-	-	Possible inhibition of PCR		

### Assay Characteristics

The analytical sensitivity of the Quantiplus® PARVO Real Time Quantitative PCR Kit is 0.2 copies/µL and 50 copies/mL.

The linear range of the Quantiplus® PARVO Real Time Quantitative PCR Kit has been determined to cover concentrations from 0.2 copies /µL to 2 x 10<sup>9</sup> copies / µL.



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