OCT 23, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3byl4qk5jvo5/v1

Protocol Citation: Come J Thieulent, Mariano Carossino, Laura Peak, Udeni B. R. Balasuriya 2023. Feline Enteric Virus Detection Assay. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4qk5jvo5/v1

MANUSCRIPT CITATION:

Protocol status: Working
We use this protocol and it's working

Feline Enteric Virus Detection Assay

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DISCLAIMER

Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

ABSTRACT

The Feline Enteric Virus Detection Assay is intended as an in vitro veterinary reagent set, based on Reverse Transcription quantitative PCR (RT-qPCR), for the detection of feline coronavirus (FCoV)/feline infectious peritonitis (FIP), feline panleukopenia virus (FPV; feline parvovirus), feline rotavirus A (FRV) and SARS-CoV-2 in rectal swabs and fecal samples.

Created: Jul 12, 2023

Last Modified: Oct 23, 2023

PROTOCOL integer ID: 84917

Funders Acknowledgement:
Vet-LIRN
Grant ID: 1 U18 FD007514

GUIDELINES

SHIPPING & STORAGE INFORMATION

The Feline Enteric Virus Detection Assay is shipped on dry ice. Reagents should arrive frozen. The Reagents in the purple and red tubes may arrive liquid, this will not result in a reduction in performance.

All reagents should be stored at -20°C upon arrival. All reagents can be stored for a minimum of one year (from the date of shipment) at -20°C without showing a reduction in performance. Positive controls should be stored at -80°C.

LIMITATIONS:

- Strict compliance with the instructions is required for optimal results.
- Appropriate specimen collection, transport, storage, and processing procedures are required for the optimal performance of this test.
- The presence of RT-PCR inhibitors may cause false negatives.
- Results of Feline Enteric Viruses Detection Assay need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL:

- The specificity of each test was validated using a panel of reference and related canine respiratory pathogens.
- The analytical sensitivity of each assay was determined using ten-fold dilution of in vitro transcribed RNA or plasmid copies number. All assays have a limit of detection (LOD95) □ 35 copies/□l.

MATERIALS

ASSAY COMPONENTS:

The reagents are assembled for 60 reactions (+ 10% extra).

Lid color	Component	Number of vials	Volume per vial
Purple	RT-PCR Master mix	1	825 µl
Red	RT mix	1	33 µl
Yellow	Primers & probes mix	1	16.5 µl
Blue	Nuclease free water	1	400 µl
Colorless	Positive Controls	2	20 µl

Table 1. Kit description.

PROBE DYE SETTING:

PROBE DYE SETTING			
TaqMan QSY™ Probe set are used as follows:			
Assays	**Pathogens**	**Reporter**	**Quencher**
FEA_1	FCOV/FIP	ABY™	QSY™
	SARS-CoV-2	FAM™	QSY™
	FRV	JUN™	QSY™
	FPV	VIC™	QSY™

Table 2. TaqMan probe set

Other Materials

- Appropriate nucleic acid extraction instrument and kits
- Appropriate real-time PCR instrument calibrated for ABY™, FAM™, JUN™ and

VIC™ dyes (e.g., Applied Biosystems 7500 Fast Real-time PCR machine)
- Vortex and benchtop centrifuge
- Appropriate 96-well reaction plate or reaction tubes with corresponding closing tape or caps
- Pipettes & tips
- Personal Protective Equipment (PPE)

Reaction Set Up

1 Thaw all reagents on ice.

2 Centrifuge all reagents on a benchtop centrifuge to ensure no liquid is in cap and keep on ice

> **Note**
>
> The Feline Enteric Virus Detection Assay does not include an internal control, but positive controls are provided. A positive and a negative control should be run simultaneously with each sample setup.

3 Setup the Master Mix according to the following table 1:

Reagents	Volume per reaction (µl)
RT-PCR Master Mix	12.5
RT mix	0.25
Primers & probes mix	1.25
Nuclease free water	6
Total volume per Master Mix	**20**
DNA/RNA template	5
Total Volume per reaction	**25**

Table 1. Reaction mix preparation

PROGRAMMING THE THERMOCYCLER

4 Select the following fluorescence channels: ABY™, FAM™, JUN™ and VIC™.

> **Note**
>
> ROX™ should be used as a passive reference dye.

5 The standard mode should be selected, following the table below:

Step	Number of cycles	Temp. (°C)	Time (min:sec)
Reverse transcription	1	50	20:00
PCR initial heat activation	1	95	15:00
Denaturation	40	94	00:45
Annealing/ extension		60#	00:75

Data acquisition

Table 2. Thermo profile

RESULTS INTERPRETATION

6 Before analysis of results, the threshold value of each fluorescent dye must be manually set in the region of exponential amplification, typically 0.1 × ΔRn value at the plateau phase.

7 Each assay is considered valid if the following criteria are met:

Assays	Pathogens	Positive Control	Negative Control
FEA_1	FCoV/FIP/ABY	Ct ≤ 22	Ct > 40
	SARS-CoV-2/FAM	Ct ≤ 22	
	FRV/JUN	Ct ≤ 22	
	FPV/VIC	Ct ≤ 22	

Table 3. Positive control validation criteria

8 The results are qualitative (Positive or Negative). A specimen is considered positive if the Ct value obtained is below the following Ct cut-off values:

Assays	Pathogens	Ct Cut-off
FEA_1	FCoV/FIP/ABY	32
	SARS-CoV-2/FAM	35
	FRV/JUN	33
	FPV/VIC	33

Table 4. Ct cut-off values