Primerdesign

R01327

Feline Diarrhoea/Gastrointestinal Diseases

Kit version: 1

Target regions

Tritrichomonas foetus: beta-tubulin 1 gene
Feline panleukopenia virus: NS1 gene
Feline coronavirus: N gene
Giardia intestinalis A-F: gdh gene
Clostridium perfringens type A: cpa gene
Cryptosporidium spp: small subunit rRNA gene

genesig®PLEX Kit

100 tests

GENESIG
Kits by Primerdesign

For general laboratory and research use only

Product Description

The genesig®PLEX Feline Diarrhoea/Gastrointestinal Diseases kit detects multiple organisms (Tritrichomonas foetus (T. foetus), feline panleukopenia virus (FPV), feline coronavirus (FCoV), Giardia intestinalis A-F (G. intestinalis), Clostridium perfringens type A (C. perfringens) and Cryptosporidium spp) which can each cause gastrointestinal infections, potentially leading to serious and potentially life-threatening complications.

Specificity

The kit is designed for the in vitro detection of T. foetus, FPV, FCoV, G. intestinalis, C. perfringens and Cryptosporidium spp genomes with a broad detection profile for these pathogens. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

The FPV primer/probe set is predicted to cross react with other members of the carnivore protoparvovirus 1 (CPPV-1) species including canine parvovirus (CPV), which would give signal in the ROX channel for tube 1.

The G. intestinalis primer/probe set is predicted to cross react with G. intestinalis G and H, which would give signal in the FAM channel for tube 2.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**Feline GI Tube 1 primer/probe mix (including IEC primer/probe mix) (100 reactions)** T. foetus (FAM), FPV (Cy5), FCoV (ROX), IEC (VIC)		**BROWN**
1	**Feline GI Tube 2 primer/probe mix (including IEC primer/probe mix) (100 reactions)** G. intestinalis (FAM), Cryptosporidium spp (Cy5), C. perfringens (ROX), IEC (VIC)		**BROWN**
1	**Feline GI Tube 1 positive control template**		**RED** (in silver foil wrapper)
1	**Feline GI Tube 2 positive control template**		**RED** (in silver foil wrapper)
4	**oasig®PLUS OneStep Lyophilised qPCR Master Mix (50 reactions per glass vial)**		**GOLD**
4	**oasig® Master Mix resuspension buffer**		**BLUE**
1	**genesig® Easy RNA internal extraction control**		**BLUE** (in gold foil wrapper)
2	**Template preparation buffer** for resuspension of internal control template and positive control template		**YELLOW**
1	**RNase/DNase free water** for resuspension of primer/probe mixes		**WHITE**

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Must be able to read fluorescence through FAM, Cy5, ROX and HEX/VIC channels.

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA/RNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

Individual primers and probes designed for each pathogen have been combined into two reactions and these can be detected through the different fluorescent channels as described in the kit contents.

The assay consists of labelled probes in one test specific for T. foetus in FAM, FPV in Cy5 and FCoV in ROX; and a second parallel test to detect G. intestinalis in FAM, Cryptosporidium spp in Cy5 and C. perfringens in ROX.

The assay includes an internal extraction control template (genesig® Easy RNA internal extraction control), which may be added to the nucleic acid extraction system (not provided) to demonstrate efficient RNA extraction, detect PCR inhibition and confirm the integrity of the PCR run. The internal extraction control assay (which is from a non-biologically relevant exogenous source) is present in both tests and the probe is labelled with the VIC fluorophore.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridise to the target cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5′-dye and a 3′-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

The kit has two positive control tubes that contain target templates. Feline GI Tube 1 positive control contains templates for T. foetus, FPV and FCoV. Feline GI Tube 2 positive control contains templates for G. intestinalis, Cryptosporidium spp and C. perfringens. Each time the kit is used, at least one positive control reaction, for each tube, must be included in the run. A positive result indicates that the primers and probes for detecting the target gene worked properly in that particular experimental scenario. If a negative result is obtained, the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Internal RNA extraction control

When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

Both the Feline GI Tube1 and Feline GI Tube 2 primer/probe mixes contain a specific primer and probe mix to detect the exogenous RNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control RNA does not interfere with detection of the target RNA even when present at low copy number. The Internal control is detected through the HEX/VIC channel and gives a Cq value of 28+/-3 depending on the level of sample dilution.

Please note: this kit targets organisms which have DNA as well as RNA genomes, but most nucleic acid extraction kits will efficiently extract both DNA and RNA.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
Feline GI Tube1 primer/probe mix (**BROWN**)	110 µl
Feline GI Tube 2 primer/probe mix (**BROWN**)	110 µl

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control RNA (**BLUE**)	600 µl
Post-PCR heat-sealed foil	
Feline GI Tube 1 positive control template (**RED**) *	500 µl
Feline GI Tube 2 positive control template (**RED**) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

4. **Resuspend the oasig®PLUS OneStep Lyophilised qPCR Master Mix in oasig® resuspension buffer, according to the table below:**

Component - resuspend in template preparation buffer	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (**GOLD**)	525 µl

Nucleic acid extraction

The internal extraction control RNA can be added either to the nucleic acid lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4 µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer.**

2. **Complete nucleic acid extraction according to the manufacturer's protocols.**

OneStep RT-qPCR detection protocol

For optimum performance and sensitivity.
All pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the OneStep amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **Prepare 2 reaction mixes, one for each primer/probe mix according to the table below: Include sufficient reactions for all samples, positive and negative controls.**

   Feline GI Tube1 reaction mix:

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (GOLD)	10 µl
Feline GI Tube1 primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4µl
Final Volume	**15 µl**

   Feline GI Tube 2 reaction mix:

Component	Volume
oasig®PLUS OneStep Lyophilised qPCR Master Mix (GOLD)	10 µl
Feline GI Tube 2 primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4µl
Final Volume	**15 µl**

2. **Pipette 15 µl of these mixes into each well according to your experimental qPCR plate set-up.**

3. **Pipette 5 µl of extracted sample into each well according to your experimental plate set-up.**
   For negative control wells use 5 µl of RNase/DNase free water (WHITE). For positive control wells use 5 µl of the positive control template (RED). The final volume in each well is 20 µl.

qPCR amplification protocol

Amplification conditions for oasig®PLUS OneStep Lyophilised qPCR Master Mix (GOLD)

	Step	Time	Temp
	Reverse transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

* Fluorogenic data should be collected during this step through the FAM, HEX/VIC, ROX and Cy5 channels

Interpretation of results

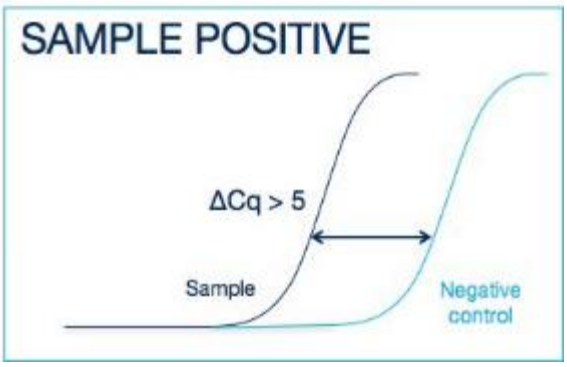
Feline GI Tube1 reaction mix:

Target (FAM/Cy5/ROX)	Internal control (VIC)	Positive control	Negative control	Interpretation
FAM+	**+ / -**	**+**	**-**	**T. FOETUS POSITIVE RESULT**
Cy5+	**+ / -**	**+**	**-**	**FPV POSITIVE RESULT**
ROX+	**+ / -**	**+**	**-**	**FCoV POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤ 35**	**EXPERIMENT FAILED** due to test contamination
+ / -	**+ / -**	**+**	**> 35**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

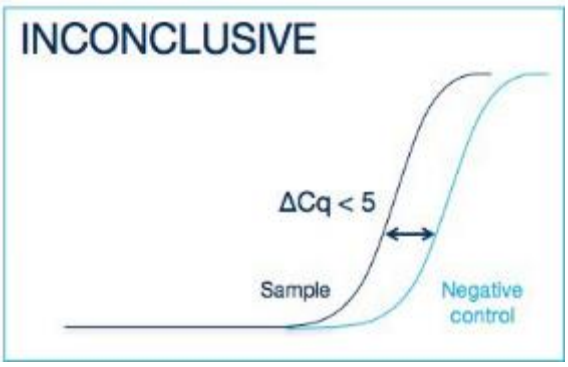
Feline GI Tube 2 reaction mix:

Target (FAM/Cy5/ROX)	Internal control (VIC)	Positive control	Negative control	Interpretation
FAM+	**+ / -**	**+**	**-**	**G. INTESTINALIS POSITIVE RESULT**
Cy5+	**+ / -**	**+**	**-**	**CRYPTOSPORIDIUM SPP POSITIVE RESULT**
ROX+	**+ / -**	**+**	**-**	**C. PERFRINGENS POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**
+ / -	**+ / -**	**+**	**≤ 35**	**EXPERIMENT FAILED** due to test contamination
+ / -	**+ / -**	**+**	**> 35**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control, then the positive sample result is invalidated, and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Positive Control

Each positive control tube contains all the templates for the targets detected by that tube and should produce positive amplification plots in the FAM, Cy5 and ROX channels. There is no internal control template within the positive control so the HEX/VIC channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each target.

The positive control is expected to amplify between Cq 16 – 23 in the FAM, Cy5 and ROX channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised and should be repeated.

No template control (NTC)

The NTC should give a flat line (flat amplification plots) through all channels. Signals in the NTC indicate cross contamination during plate set up.

RNA Internal extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of nucleic acid in the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data

Presence of the target pathogens is shown by amplification in the channels indicated in the kit contents section. Positive signals indicate positive tests for those targets. It may be possible for samples to contain multiple targets, therefore positive results in the FAM, Cy5 and ROX channels may be present.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period, Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee is expired.

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