ViPrimePLUS Feline Leukaemia Virus RT-qPCR Kit

Quantitative assay for real-time RT-PCR detection of Feline Leukaemia Virus genome

Product Code: QV2010
Pack Size: 150 reactions

INTENDED USE
The ViPrimePLUS Feline Leukaemia Virus RT-qPCR Kit is a Taqman probe-based real-time PCR assay for the detection of Feline Leukaemia Virus genome in blood and bodily fluid samples. This assay is intended for research use only.

INTRODUCTION
Feline Leukaemia Virus (FeLV) is an oncogenic retrovirus that infects cats and causes leukaemia. It is often found in cats positive for feline immunodeficiency virus (FIV). There are 4 subgroups of FeLV; 1) Subgroup A which is found in naturally infected cats and tends to be less pathogenic, 2) Subgroup B which increases neoplastic development frequency, 3) Subgroup C which accounts for 1% of the infected cats, causes severe anemia and 4) Subgroup T which leads to lymphoid depletion and immunodeficiency. It is believed that subgroups C and D arise as the mutated form of subgroup A. The virus may be transmitted vertically (in utero or by milk) or horizontally (by secretions and excretions). Initial infection may be limited to lymphoid tissue but as second stage progresses, the virus may be circulated to the bone marrow, oesophagus, bladder, respiratory tract and salivary glands. Once infection reaches the bone marrow, mortality rates can raise up to 50-80%. The disorders due to the FeLV infections include immunosuppression, lymphoid tumour, anemia, reproduction problems and enteritis.

PRINCIPLE OF TEST
The kit contains primers and Taqman® probe that target the U3 region LTR. In this one-step real-time RT-PCR, reverse transcription of this viral RNA is combined with the qPCR step in a single tube reaction. This closed-tube assay reduces the chances of contamination and improves the sensitivity of the test.

Based on the Taqman® probe detection principle, the 5'-reporter dye and 3'-quencher dual-labelled oligonucleotide (Taqman® probe) hybridizes on a specific region within the amplified fragment. During amplification, the probe is cleaved and the reporter dye (fluorophore) is released. The fluorescent signal intensity detected is proportional to the number of amplicons. The Ct value (the cycle at which the rise of fluorescent signal from the baseline is first significant) is used for quantification purposes. Target pathogen amplification is detected using FAM channel.

The kit provides the Internal Extraction Control (IEC) as inhibition control. During nucleic acid extraction protocol, IEC RNA template is added in the lysis stage. An IEC specific primers and probe labelled with a different dye is provided to be run in the same reaction with the pathogen-specific primers and probe mix. The IEC amplicons are detected via VIC/HEX channel at Ct value 28±3 depending on the sample dilution.

A positive control with known copy number is provided for standard curve construction and absolute quantification. It can also be used at a single dilution for qualitative analysis control of the experimental set-up. Extra care must be taken to avoid cross-contamination.

QUALITY CONTROL
Each lot of ViPrimePLUS Feline Leukaemia Virus RT-PCR Kit has been tested against predetermined specifications to ensure consistent product quality under ISO 9001:2008 – certified Quality Management System.

SENSITIVITY & SPECIFICITY
The detection limit is tested to 100 copies per reaction. The primers and probe are 100% specific.

STORAGE & STABILITY
Store at -20°C and avoid light exposure. Stable at -20°C up to the expiry date stated. Keep in aliquot to reduce freeze-thaw cycles.

LIMITATION OF TEST
For research use only. Not recommended for diagnosis of disease in humans or animals.
Result is dependent on the yield and quality of the nucleic acids extracted from the method of extraction. Thus, it is important to do spectrophotometric and gel analysis on the extracted samples.

KIT COMPONENTS

FeLV Primers and Probe Mix (FeLV PPM)	Tube
Positive Control	Tube
Internal Extraction Control Primers and Probe Mix (IEC PPM)	Tube
Internal Extraction Control (IEC RNA)	Tube
Nuclease Free Water	Tube
Template Preparation Buffer	Tube

SAMPLE MATERIAL
The kit is suitable for RNA extracted by most commercial kits, provided the purity, concentration and integrity are within acceptable range. IEC is provided to eliminate doubts of PCR inhibition. Suitable sample types are blood and bodily fluids.

PROTOCOLS

Reconstitution of reagents

*Pulse-spin each tube prior to opening.

Components	Volume	Reagents
FeLV PPM (Amber)	165µl	Nuclease Free Water
IEC PPM (Amber)	165µl	Nuclease Free Water
IEC RNA	600µl	Nuclease Free Water
Positive Control Template	500µl	Template Preparation Buffer

RNA extraction

Add 4µl of IEC RNA into each sample suspended in the lysis/extraction buffer.
**DO NOT* add IEC directly into unprocessed biological sample.*

Real-time PCR reaction set-up

Recommended real-time PCR reaction set-up:

Reagents	1 reaction (µl)
2x RT-PCR Mastermix	10
FeLV PPM	1
IEC PPM (not required when preparing standards' reaction mix)	1
Nuclease-free water	3
Sample RNA	5

**Suggested sample concentration 5-20ng/µl.*
**Adjust nuclease-free water to make up the final reaction volume of 20µl.*
**Prepare an extra reaction to accommodate for pipetting error.*

Negative control

Set aside one tube/well as negative control where 5µl of nuclease-free water is used as the template. This is also known as no template control.

Positive control

For qualitative analysis, set aside one tube as positive control where 5µl of the positive control is used as the template.

Positive control (optional)

For quantitative analysis only, prepare 6 serially diluted positive control templates for standard curve construction.

a) Pipette 90µl of nuclease-free water into 5 clean microtubes and label 2-6 accordingly.
b) Pipette 10µl of Positive Control into tube 2.
c) Vortex thoroughly and spin down.
d) Change pipette tip and pipette 10µl from tube 2 to tube 3. Vortex and spin down.
e) Repeat steps d) to complete the dilution series.
f) Pipette 5µl of standard template into each tube/well accordingly.

Standards	Copy number/µl
Tube 1 Positive Control	2×10^5
Tube 2	2×10^4
Tube 3	2×10^3
Tube 4	2×10^2
Tube 5	20
Tube 6	2

Set the thermal cycler parameters as follows:

Step	Time	Temp	Cycles	Scan
Reverse transcription	10mins	55℃		
Enzyme activation	8mins	95℃		
Denaturation	10secs	95℃	50	
Anneal/Elongation	1min	60℃		√*

*FAM or VIC/HEX

INTERPRETATION OF RESULTS

Pathogen specific amplification signal is detected via FAM channel, while IEC amplification is detected via VIC/HEX channel. The signal is positive if the amplification curve crosses the threshold line. The result is relevant provided both positive and negative controls give valid results.

Summary of interpretation:

Target	IEC	Negative Control	Positive Control	Interpretation
+	+	-	+	Valid, positive
+	-	-	+	Valid, positive
-	+	-	+	Valid, negative
-	-	-	-	Invalid
+	+	+	+	Invalid

Internal Extraction Control

When used accordingly and assuming 100% extraction efficiency, a Ct value of 28±3 is within normal range. A high Feline Leukaemia Virus genome copy amplification may out compete the IEC amplification. Thus, the latter may not produce an amplification signal. The positive result is still valid in this case.

TROUBLESHOOTING

Problem	Possibility	Suggestion
Negative control / No template control gives positive result	Carry over contamination	Change nuclease-free water. Use fresh aliquots of reagents. Use filtered tips. Load positive control last.
No signal detected from positive control	Incorrect programming of instrument	Check program.
	Reagents expired	Check the expiry date of reagents before repeat.
	Storage condition not complying with instructions	Check storage condition properly and store at correct storage condition to avoid the degradation of reagents.
	Pipetting error	Pipette the correct volume of reagents to reconstitute the components of kit and mix well.
Internal extraction control does not give a signal in apparently negative samples	Inhibitors in the samples extracted	Repeat the extraction.
	Low recovery of RNA extracted	Repeat the extraction by enlarge the sample size.
	IEC added directly into unprocessed biological sample – lead to degradation and loss of signal	Add IEC into each sample suspended in the lysis/extraction buffer.

DEVIATION OF MASTERMIX FORMULATION

Manufacturers use varying methods to calibrate a real-time PCR reaction. For this reason, we provide several Mastermix formulations for those platforms.

Master Mix	Compatible Hardware
Original	Biometra qTower, Cepheid SmartCycler®, Eppendorf Mastercycler, Fluidigm BioMark™, Illumina Eco, MJ Chromo4, Opticon, PCRMax Eco™, Roche lightcycler® 480, lightcycler® LC96 and lightcycler® Nano Platforms, RotorGene, Thermo PikoReal™
Low Rox (-LR)	Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7.
Rox (-R)	Applied Biosystems 7000, 7300, 7700, 7900 and 7900HT FAST platforms, GeneAmp® 5700, StepOne™, StepOne™ PLUS
iCycler platform (-iC)	BioRad iCycler, IQ4 AND IQ5 platforms
Stratagene platform (-SG)	Stratagene MX, MX4000P®, MX3000P® and MX3005® platforms
Capillary lightcyclers (-CL)	Roche Capillary Lightcycler 1.0-2.0.

WARRANTY & LIMITED LIABILITY

The performance characteristics stated were obtained using the assay procedure in this insert. Failure to comply with the instructions may derive inaccurate results. In such event, manufacturer disclaims all warranty expressed, implied or statutory including the implied warranty of merchantability and the fitness of use.

The manufacturer will not be liable for any damage caused by misuse, improper handling and storage; non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.