Feline Immunodeficiency Virus RT-qPCR
FIV 202

DESCRIPTION

The **FIV dtec-RT-qPCR** comprises a series of specific targeted reagents designed for **Feline immunodeficiency virus** detection by using qPCR. Feline immunodeficiency virus is a single-stranded negative-sense RNA virus belonging to the *Lentivirus* genus. FIV is the only non-primate lentivirus to cause an AIDS-like syndrome, attacking the immune system, but is not typically fatal for cats. FIV is transmitted primarily through saliva, mainly bites, and progresses through similar stages to HIV in humans. After the initial stage of infection, there are an asymptomatic stage of variable length of time. Some cats stay in this latent stage for only a few months, but for some it can last for years. Finally, the cat progresses into the final stage, wherein the cat is extremely susceptible to secondary diseases that inevitably are the cause of death.

PRINCIPLE OF THE METHOD

Polymerase chain reaction (PCR) allows the amplification of a target region from a DNA template by using specific oligonucleotides. In real-time reverse transcription PCR (RT-qPCR), the RNA is first transcribed to complementary DNA by a reverse transcriptase. The accumulating amplified product can be detected at each cycle with fluorescent dyes. This increasing signal allows to achieve sensitive detection and quantification of pathogens.

KIT CONTENT

TargetSpecies dtec-RT-qPCR-mix (AMBER TUBE), contains a mixture of specific forward/reverse primers and probe, at optimal concentration lyophilized after synthesis. 100 reactions

Resuspension buffer (WHITE CAP), 130 µl

DNase/RNase free water (GREEN CAP), 1.5 ml

[OPTIONAL] Internal Control qPCR-mix (AMBER TUBE), contains a mixture of primers, probe and the DNA template to achieve a suitable internal control of PCR. 100 reactions

GPS™-mix-RT (BLUE CAP), it is a 4X mastermix containing a polymerase, retrotranscriptase, dNTPs and buffer. 500 µl, 100 reactions

Standard Template (RED CAP), dehydrated target copies for positive control.

Template buffer (BLACK CAP), exclusive for resuspension of the Standard Template. 150 µl

STORAGE CONDITIONS

All the components of **FIV dtec-RT-qPCR** are stable at room temperature for transport. At arrival, if not immediately used, it should be stored at -20 ºC. The product is stable for one year (see expiration date on the label).

Once **TargetSpecies dtec-RT-qPCR-mix** (AMBER TUBE) has been dissolved (with or without Internal Control), we recommend splitting the content in several aliquots and store at -20 ºC to avoid freezing-thawing cycles which may reduce assay sensitivity.

GPS™-mix-RT (BLUE CAP) is stable at room temperature for transport but should be stored at -20 ºC at arrival.

For **Standard Template** (RED CAP), once dissolved, we recommend storing **in an exclusive box** at -20 ºC.

MATERIALS REQUIRED BUT NOT PROVIDED

- RNA isolation kit (*GPSpin* extraction/purification kits recommended)
- DNase/RNase free water (to prepare standard curve dilution)
- Micropipettes and sterile pipette tips with filters
- Low retention tubes certified as DNase/RNase free
- qPCR tubes, strips or plates
- Vortex mixer and spinner centrifuge
- Cooling block
- Real-time PCR device

WARNINGS AND PRECAUTIONS

- ⓘ For Research Use Only (RUO).
- ⓘ To avoid possible misuse, carefully read the handbook.
- ⓘ Proper training is recommended for correct operation of the kit.
- ⓘ According to good laboratory practices, always wear a suitable lab coat, disposable gloves, and protective goggles.
- ⓘ All the instruments used must been verified and calibrated according to the manufacturer's recommendations.
- ⓘ Any kind of sample can be analysed with this assay after appropriate nucleic acid extraction.

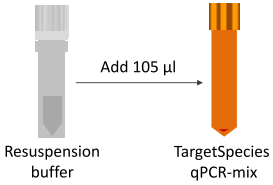
General precautions

- ⚠ To prevent contamination of micropipettes, use sterile tips with filters.
- ⚠ Micropipettes used to dilute the Standard Template should not be used for other PCR reagents.
- ⚠ Extract, store and prepare positive materials (samples, positive controls and PCR products) in a separately laboratory environment.
- ⚠ To decrease the risk of contamination, we recommend that all pipetting be performed in a PCR clean environment. Ideally, this would be a designated PCR lab or PCR cabinet.
- ⚠ To avoid cross-contamination with the positive control, pipette it after closing reaction tubes with negative control and samples.
- ⚠ The workflow in the laboratory should be unidirectional, from clean preamplification area to the amplification area.
- ⚠ Keep components refrigerated in a cooling block.
- ⚠ Protect the primer/probe from prolonged exposure to light.
- ⚠ For dsRNA virus preincubate sample at 95 ºC for 5 minutes.

RESUSPENSION PROTOCOLS

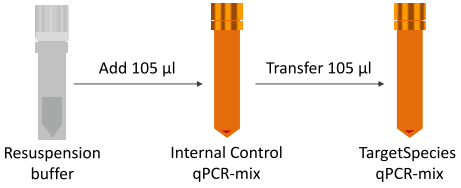
Resuspension of TargetSpecies dtec-RT-qPCR-mix

For reconstitution of **TargetSpecies dtec-RT-qPCR-mix** (AMBER TUBE) and before opening, pulse-spin the tube in a centrifuge to ensure that lyophilized product goes down and will not be spilt. Add 105 µl of **Resuspension buffer** (WHITE CAP), vortex the tube thoroughly (or pipette the solution up and down on the sides of the tube), and harvest by pulse-spin in the centrifuge. Use 1 µl of this suspension in PCR reactions with a final volume of 20 µl.



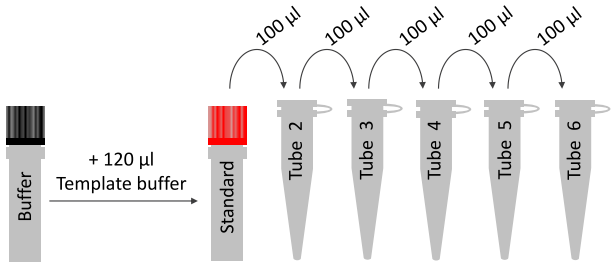
Resuspension of TargetSpecies dtec-RT-qPCR-mix with Internal Control

For reconstitution of **Internal Control qPCR-mix** (AMBER TUBE) and **TargetSpecies dtec-RT-qPCR-mix** (AMBER TUBE) and before opening, pulse-spin the tubes in a centrifuge to ensure that lyophilized product goes down and will not be spilt. Add 105 µl of **Resuspension buffer** (WHITE CAP) to the **Internal Control qPCR-mix** (AMBER TUBE), vortex the tube thoroughly (or pipette the solution up and down on the sides of the tube), and harvest by pulse-spin in the centrifuge. Transfer the volume to the **TargetSpecies dtec-RT-qPCR-mix** (AMBER TUBE), vortex the tube thoroughly and harvest by pulse-spin in the centrifuge. Use 1 µl of this suspension in PCR reactions with a final volume of 20 µl.



PREPARATION OF STANDARD CURVE DILUTION SERIES

1) Pipette 900 µl of **DNAse/RNAse free water** (not provided) into five tubes and label as 2 to 6
2) Pulse-spin the **Standard Template** (RED CAP), reconstitute with 120 µl of **Template buffer** (BLACK CAP) and vortex thoroughly, label as num. 1
3) Pipette 100 µl of diluted **Standard Template** (RED CAP), into tube 2
4) Vortex thoroughly and pulse-spin
5) Change tip and pipette 100 µl from tube 2 into tube 3
6) Vortex thoroughly and pulse-spin
7) Repeat steps 5 and 6 with the tubes 4 to 6 to complete serial dilution



Standard curve dilution series	copies/µl	copies in 5 µl
Standard Template (RED CAP)	2×10^5	10^6
Tube 2	2×10^4	10^5
Tube 3	2×10^3	10^4
Tube 4	2×10^2	10^3
Tube 5	2×10	10^2
Tube 6	2	10

Pipette 5 µl of template into each well for the standard curve according to your plate set-up. The final volume in each qPCR reaction well is 20 µl.

PCR SET-UP PROTOCOL

It is advisable to prepare a reaction pre-mix in a nuclease-free tube on a cooling block, according to the following table. Prepare enough reaction mix for the desired number of reactions, for your samples and the positive/negative controls considered for your experiment. Reverse transcription is achieved in the same tube with a One-Step protocol to prevent contamination, reduce errors, and save significant materials and time.

Reagent	Volume
GPS™-mix-RT (BLUE CAP)	5 µl
TargetSpecies dtec-RT-qPCR-mix (AMBER TUBE)	1 µl
DNase/RNase free water (GREEN CAP)	9 µl
Reaction pre-mix volume	15 µl

Once prepared, pipette 15 µl of this reaction mix into each well or PCR tube according to your experimental platform.

Add 5 µl of samples or diluted **Standard Template** (RED CAP) to each PCR tube to reach a final PCR volume of 20 µl.

Reagent	Volume
Dispensed reaction pre-mix volume	15 µl
Sample (or DNase/RNase free water)[1]	5 µl
FINAL REACTION VOLUME	**20 µl**

1 In the case of negative control, add 5 µl of DNase/RNase free water (GREEN CAP) instead of sample (see Recommended Reaction Controls section).

AMPLIFICATION REGIME

Once all reactions mixes are ready and gently closed, vortex the tube thoroughly, harvest by pulse-spin in the centrifuge, and place plate or tubes in the block/rotor of the thermocycler programmed to run the cycling regime described on the table. Take into account that the fluorogenic signal must be collected by using the FAM channel for the target. If the Internal Control is added use the HEX channel. Probe includes a Dark Quencher which can be set up as any non-fluorescent quencher (NFQ) in the software.

	Step	Time	Temperature
	Retrotranscription	10 min	50 ºC
	Activation	2 min	95 ºC
40 Cycles	Denaturation	5 sec	95 ºC
	Hybridization / Extension and data collection[1]	20 sec	**60 ºC**

1 Fluorogenic signal should be collected during this step by using the **FAM** channel for the target and by using the **HEX** channel for the Internal Control.

GPS™ reagents are compatible with all qPCR devices. The use of a passive dye as ROX is not required.

RECOMMENDED REACTION CONTROLS

These qPCR reaction controls are recommended when considering the guidelines of ISO/IEC 17025 Standard. When setting-up your qPCR protocol, select the controls considered better suits your quality system.

Negative Control (Ctrl -): Add 5 µl of DNase/RNase free water (GREEN CAP) to 15 µl of reaction mix. Accordingly, this reaction should be negative. A positive result may be considered as a symptom of contamination in some reagents of reaction mix, making the test inconclusive. Reagents must be discarded.

Positive Control (Ctrl +): Prepare a standard curve dilution series as described above from the **Standard Template** (RED CAP). Add 5 µl of the Standard Template dilution (i.e., 2 x 10^2 copies/µl; Tube 4) to 15 µl of the reaction mix. A positive result indicates that qPCR setup is correct and works. If negative, the test should be carefully repeated after checking the thermal protocol.

Sample Inhibition Control (sIC): We recommend to run reactions in parallel to test possible inhibition effects of compounds (inhibitors) present in the sample matrix. Simply, to a reaction tube, add a known amount of **Standard Template** (RED CAP) (i.e., 2 x 10^2 copies/µl) together with the sample.

Reagent	Volume
GPS™-mix-RT (BLUE CAP)	5 µl
TargetSpecies dtec-RT-qPCR-mix (AMBER TUBE)	1 µl
Standard Template dilution (i.e., 2 x 10^2 copies/µl)[1]	5 µl
DNase/RNase free water (GREEN CAP)	4 µl
Sample	5 µl
FINAL REACTION VOLUME	**20 µl**

1 Tube 4 of the curve dilutions series obtained from **Standard Template** (RED CAP).

An optimal result should show a positive signal, equal or higher (same or lower Ct) than these found for the Positive Control alone. Inhibition may be total (negative result) or partial, observing a considerable increase in the Ct when compared to this of the Standard Template dilution added. If inhibition is observed, a sample dilution to 1/10 may be recommended (if concentration is not close to detection limit). The sample inhibition control is external, allowing to check the inhibition on the main target of interest.

Extraction Negative Control (ExtCtrl -): Perform an extraction according to your extraction protocol without addition of sample. Add 5 µl of extraction negative sample to 15 µl of reaction mix. In this case, the test includes the reagents used in the extraction steps. If positive, when the **Negative Control** is negative, a contamination occurs during the extraction process. Extraction reagents must be discarded.

Extraction Positive Control (ExtCtrl +): Perform an extraction according to your extraction protocol adding the **Standard Template** (RED CAP), or nucleic acid material extracted from pure cultures into the first extraction buffer. The positive extraction control would include the effectiveness of the extraction method used. A positive result should be expected. If negative, extraction must be carefully repeated or the extraction method replaced.

INTERPRETATION OF RESULTS

The linear regression obtained from the logarithm of the copy number versus Ct gives both constants Y-intercept and slope of the standard curve (equation 1). The number of copies in the sample can be calculated based on the regression (equation 2).

$$Ct = Y\ intercept + Slope\ x\ log(copy\ number) \quad (1)$$

$$Copy\ number = 10^{\frac{(Ct - Y\ intercept)}{Slope}} \quad (2)$$

To obtain the sample quantification directly from the device, the Standard dilution series must be defined in the software of your qPCR device as Standard with the specified copies for each dilution (see PREPARATION OF STANDARD CURVE DILUTION SERIES). Standard curve can be defined as total copy number or copies/µl.

To refer the values obtained with qPCR to the sample material, please take into account the elution volume after extraction, the sample volume processed, and any dilution performed.

Ctrl -	Ctrl +	Sample	IC / sIC	ExtCtrl -	Interpretation
+	+ / -	+ / -	+ / -	+ / -	PCR reagents contaminated
-	-	+ / -	+ / -	+ / -	Experiment fails
-	+	+ / -	+ / -	+	Contamination at extraction step
-	+	-	+	-	**Negative sample**
-	+	+	+ / -	-	**Positive sample**
-	+	-	-	-	PCR inhibition

Key symbols + and - : amplification does or not occur, respectively.

VALIDATION METHODS

All batches are calibrated with a standard curve from 10^6 to 10 copies with our Standard Template. Diverse parameters are evaluated: Ct, slope, R^2 and efficiency. All this information is available in the **Quality Certification** provided to the customer by GPS™.