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Feline Leukemia Virus RT-PCR Detection Kit — Product Insert

Product # 44000

Pathogen Information

Feline leukemia virus (FeLV) is a retrovirus similar to the feline immunodeficiency virus (FIV). Approximately 2 to 3% of all cats are infected with FeLV. Although many of the symptoms caused by FeLV and FIV are similar, the specific ways in which these diseases are caused differs. Cats persistently infected with FeLV serve as a source of infection. The virus is shed in high amounts in saliva and nasal secretions as well as in urine, feces and milk. As a result, cat-to-cat transfer of virus may occur from bite wounds, during mutual grooming and also via shared use of litter boxes or feed dishes. In addition, unlike FIV infection, FeLV-infected cats usually demonstrate various forms of symptomatic illnesses including loss of appetite, progressive weight loss, poor coat condition as well as infections of the skin, urinary bladder and upper respiratory tract. The virus could become fatal if it overcomes the feline immune system during the later stage of infection. Hence, the rapid, early diagnosis of FeLV-infected cats is the only reliable way to prevent exposure to the virus and help to prevent the spread of this disease.

Principle of the Test

Norgen's FeLV RT-PCR Detection Kit constituents a ready-to-use system for the isolation and detection of FeLV using end-point one-step RT-PCR. The kit first allows for the isolation of FeLV RNA from blood or swabs using spin-column chromatography based on Norgen's proprietary resin. The FeLV RNA is isolated free from inhibitors, and can then be used as the template in a one step RT-PCR reaction for FeLV detection using the provided FeLV Detection Mastermix. The FeLV Detection Mastermix contains reagents and enzymes for the specific amplification of a 310 bp region of the viral genome. In addition, Norgen's FeLV RT-PCR Detection Kit contains a second Mastermix, the RT-PCR Control Master Mix, which can be used to identify possible PCR inhibition and/or inadequate isolation via a separate RT-PCR reaction with the use of the provided *PCR control (PCRC)* or *Isolation Control (IsoC),* respectively. This kit is designed to allow for the testing of 24 samples.

Kit Components:

Component	Contents
Lysis Solution	30 mL
Wash Solution	11 mL
Elution Buffer	2 mL
Mini Spin Columns	24
Collection Tubes	24
Elution tubes (1.7 mL)	24
2x FeLV Detection RT-PCR Mastermix	0.35 mL
2x RT-PCR Control Mastermix	0.35 mL
Isolation Control (IsoC)*[a]	0.3 mL
FeLV Positive Control (PosC)*[b]	0.1 mL
Nuclease Free-Water	1.25 mL
Norgen's RNA Marker	0.1 mL
Product Insert	1

** IsoC = Isolation Control ; PosC= Positive Control*
[a] The isolation control is a cloned RNA transcript.
[b] The positive control is FeLV RNA transcript

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 95-100% ethanol
- Thermocycler and or Real-Time PCR System
- Micropipettes with an accuracy range between 1-10 µL, 10-100 µL and 100-1000 µL
- Laminar flow hood for extractions
- Vortex
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- Microcentrifuge tube rack
- Disposable latex gloves
- β-mercaptoethanol

Storage Conditions and Product Stability

- The Positive Control (**FeLV *PosC,*** red cap) and Isolation Control (***IsoC*,** orange cap) should be stored at -70°C. If needed, make aliquots of the controls according to the volume used in the protocol (10 µL of **FeLV *PosC*** or 10 µL of ***IsoC***) prior to freezing.
- The **2X FeLV Detection RT-PCR Mastermix** and the **2X RT-PCR Control Mastermix** should be stored at -20°C upon receipt (-70°C for long-term). Make appropriate aliquots and store at -20°C if needed.
- All other kit components may be stored at room temperature
- The **2X FeLV Detection RT-PCR Mastermix** and the **2X RT-PCR Control Mastermix**, Positive Control and Isolation Control should not undergo repeated freeze-thaw (a maximum freeze-thaw of three times).
- For RT-PCR:
    - Allow reagents to thaw at room temperature prior to use
    - When thawed, mix the components and centrifuge briefly
    - Work quickly on ice
    - After addition of RT-PCR Mastermix use within one hour

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's FeLV RT-PCR Detection Kit, including the 2x FeLV Detection RT-PCR Mastermix, 2X RT-PCR Control Mastermix, FeLV Isolation Control and FeLV Positive Control are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's FeLV RT-PCR Detection Kit is designed for research purposes only.

Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Disclaimers

The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

General Precautions

- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Wear personal protective equipment, including gloves and lab coats when handling kit reagents. Wash hands thoroughly when finished performing the test.
- Dispose of unused kit reagents and specimens according to local, provincial or federal regulations.
- Workflow in the laboratory should proceed in a uni-directional manner, beginning in the pre-amplification area(s) (i.e. specimen collection and RNA extraction) and moving to the amplification / detection area(s) (RT-PCR and gel electrophoresis).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and sample preparation. No cross-movement should be allowed between the different areas.
- Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only.
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water.
- Clean all surfaces with commercially available RNase decontamination solutions.
- When working with purified RNA samples, ensure that they remain on ice during downstream applications.

INSTRUCTIONS FOR USE

Important Notes Prior to Beginning Protocol:

- Bodily fluid (blood and saliva) of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood or saliva.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.

- A variable speed microcentrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 25 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 36 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required. β-mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is important to work quickly during this procedure.
- **FeLV Isolation Control (*IsoC*)**
    - A FeLV Isolation Control (***IsoC***) is supplied. This allows the user to control the RNA isolation procedure. For this assay, add the FeLV Isolation Control *(**IsoC**)* to the lysate during the isolation procedure
    - The FeLV Isolation Control *(**IsoC**)* must not be added to the sample material directly.
    - Do not freeze and thaw the FeLV Isolation Control *(**IsoC**)* more than 2 times.
    - The FeLV Isolation Control *(**IsoC**)* must be kept on ice at all times during the isolation procedure.
- The RT-PCR components of the FeLV RT-PCR Detection Kit should remain at -20°C until RNA is extracted and ready for RT-PCR amplification.
- Acceptable specimen types include blood or nasal/throat swabs.
- If using swabs, use only sterile Dacron, nylon or rayon swabs with plastic shafts. Note: Do not use calcium alginate swabs as they may contain substances that are inhibitory to PCR.
- It is recommended that no more than 100 µL of blood be used in order to prevent clogging of the column.
- We recommend the use of this kit to isolate RNA from non-coagulating fresh blood using EDTA or heparin as the anti-coagulant.
- This kit is also compatible with samples collected using Norgen's Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100). Please follow the instructions provided with that kit for specimen collection and preservation.
- FeLV has a poor survival rate outside the infected body. It is important to add the **Lysis Solution** to the specimen as soon as possible (within 6 hours) or collect the specimen using Norgen's Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100).
- It is important to work quickly during this procedure.

A. SPECIMEN COLLECTION AND LYSATE PREPARATION

i. Blood Lysate Preparation:

1) Add 350 µL of the **Lysis Solution** to an RNase-free microcentrifuge tube.
2) Add up to 100 µL of blood. Vortex for 10 seconds to mix.

   Note: FeLV has a poor survival rate outside the infected body. It is important to add the **Lysis Solution** to the specimen as soon as possible (within 6 hours). In the presence of the Lysis Solution components, the virus could be stable for hours if stored at room temperature and > 1 month if stored at -70°C.

3) Add 10 µL of the Isolation Control (***IsoC***) to the lysate. Vortex for 10 seconds to mix.
4) Add 200 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
5) Proceed to RNA Isolation (Step B).

ii. Lysate Preparation from Specimens previously collected using Norgen's Sample Collection Kit For Upper Respiratory Tract Infectious Agents (Cat #29100)

1) Transfer 300 µL of preserved specimen to an RNase-free microcentrifuge tube.
2) Add 300 µL of the **Lysis Solution** and vortex for 10 seconds to mix

3) Add 10 µL of the Isolation Control (***IsoC***) to the lysate. Vortex for 10 seconds to mix.
4) Add 300 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
5) Proceed to RNA Isolation (Step B).

iii. Lysate Preparation Directly from Swab:

1) Nasal/throat swabs can be placed directly into an RNase-free microcentrifuge tube containing 1 mL of the **Lysis Solution**.
2) Using sterile techniques, cut the tip where the nasal or throat cells were collected and place into microcentrifuge tube containing the **Lysis Solution**.
3) Close the tube and vortex for 1 minute to release the virus particles.
4) Using a sterile pipette transfer 400 µL of the lysate into another RNase-free microcentrifuge tube.

   Note: FeLV has a poor survival rate outside the infected body. It is important to add the **Lysis Solution** to the specimen as soon as possible (within 6 hours). In the presence of the Lysis Solution components, the virus could be stable for hours if stored at room temperature and > 1 month if stored at -70°C.

5) Add 10 µL of the Isolation Control (***IsoC***) to the lysate. Vortex for 10 seconds to mix.
6) Add 200 µL of 95% ethanol to the lysate. Vortex for 10 seconds to mix.
7) Proceed to RNA Isolation (Step B).

B. SPECIMEN RNA PURIFICATION

Following the lysate preparation, viral RNA can be extracted from the patient specimens using the supplied buffers and solutions according to the following protocol:

1. Assemble a column with one of the provided collection tubes.
2. Apply the lysate with ethanol (up to 650 µL) to the column and centrifuge for 1 minute at 14,000 rpm.

   Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed through, spin for an additional minute.

3. Discard the flowthrough and reassemble the spin column with its collection tube.
4. Depending on lysate volume, repeat steps **B2** and **B3**.
5. Apply 400 µL of **Wash Solution** and centrifuge for one minute at 14,000 rpm.

   Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed through, spin for an additional minute.

6. Discard the flowthrough and reassemble the spin column with its collection tube.
7. Repeat steps **B5** and **B6** two more times (for a total of 3 washes).
8. Spin the column for 2 minutes to thoroughly dry the resin at 14,000 rpm. Discard the collection tube.
9. Place the column into a new 1.7 mL Elution tube.
10. Add 50 µL of **Elution Solution** to the column.
11. Centrifuge for 2 minutes at 2,000 rpm followed by a 2 minute spin at 14,000 rpm. Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column for an additional minute at 14,000 rpm.
12. The purified RNA sample could be used immediately for RT-PCR as described below. It is recommended that samples be placed at -70°C for long term storage.

C. FeLV RT-PCR Assay Preparation

Notes:

- Before use, suitable amounts of all RT-PCR components should be completely thawed at room temperature, vortexed and centrifuged briefly.
- The amount of **2X FeLV Detection RT-PCR Mastermix** and **2X RT-PCR Control Mastermix** provided is enough for up to 32 RT-PCR reactions (24 sample RT-PCR, 4 positive control RT-PCR and 4 no template control RT-PCR) each.
- For each sample, one RT-PCR reaction using the **2X FeLV Detection RT-PCR Mastermix** and one RT-PCR reaction using **2X RT-PCR Control Mastermix** should be set up in order to have a proper interpretation of the results.
- For every RT-PCR run, one reaction containing FeLV Positive Control (**FeLV *PosC***) and one reaction as no template control must be included for proper interpretation of results.
- The recommended minimum number of RNA samples tested per RT-PCR run is 6.
- Using a lower volume from the sample than recommended may affect the sensitivity of FeLV Limit of Detection.

1. Prepare the RT-PCR reaction for sample detection (Set #1, using **2X FeLV Detection RT-PCR Mastermix**) and the RT-PCR reaction for control detection (Set #2, using **2X RT-PCR Control Mastermix**) as shown in Table 1 below. The recommended amount of sample RNA to be used is 2.5 µL. However, a volume between 1 and 5 µL of sample RNA may be used as template. Ensure that one FeLV detection reaction and one control reaction is prepared for each RNA sample. Adjust the final volume of the RT-PCR reaction to 20 µL using the Nuclease-Free Water provided.

Table 1. RT-PCR Assay Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
2X FeLV Detection RT-PCR Mastermix Or 2X RT-PCR Control Mastermix	**10 µL**
Sample RNA	**2.5 µL**
Nuclease-Free Water	**7.5 µL**
Total Volume	**20 µL**

2. For each RT-PCR set, prepare **one** positive control RT-PCR as shown in Table 2 below:

Table 2. RT-PCR Positive Control Preparation

RT-PCR Components	Volume Per RT- PCR Reaction
2X FeLV Detection RT-PCR Mastermix Or 2X RT-PCR Control Mastermix	**10 µL**
FeLV *Positive Control (PosC)*	**10 µL**
Total Volume	**20 µL**

3. For each RT-PCR set, prepare **one** no template control RT-PCR as shown in Table 3 below:

Table 3. RT-PCR Negative Control Preparation

RT-PCR Components	Volume Per RT-PCR Reaction
2X FeLV Detection RT-PCR Mastermix Or 2X RT-PCR Control Mastermix	**10 µL**
Nuclease-Free Water	**10 µL**
Total Volume	**20 µL**

Therefore, at a minimum, each PCR run will contain 6 separate RT-PCR reactions.

C. One-Step RT-PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 4 below.
2. Run one-step RT-PCR.

Table 4. FeLV Assay Program

One Step RT-PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**50°C**	**25 min**
Cycle 2	**Step 1**	**95°C**	**5 min**
Cycle 3 (35x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**
Cycle 4	**Step 1**	**72°C**	**5 min**
Cycle 5	**Step 1**	**4°C**	**∞**

D. FeLV One Step RT- PCR Assay Results Interpretation

1. For the analysis of the RT-PCR data, the entire 15-20 µL RT-PCR Reaction should be loaded on a 1X TAE 1.7% Agarose RNA gel along with 10 µL of Norgen's RNA Marker (provided). Prepare enough agarose gel for running one set of RT-PCR of FeLV detection and one set of RT-PCR for controls detection.
2. The RT-PCR products should be resolved on the 1X TAE 1.7% Agarose gel at 150V for 30 minutes (Gel running time will be vary depending on an electrophoresis apparatus).
3. Sample results are provided below:

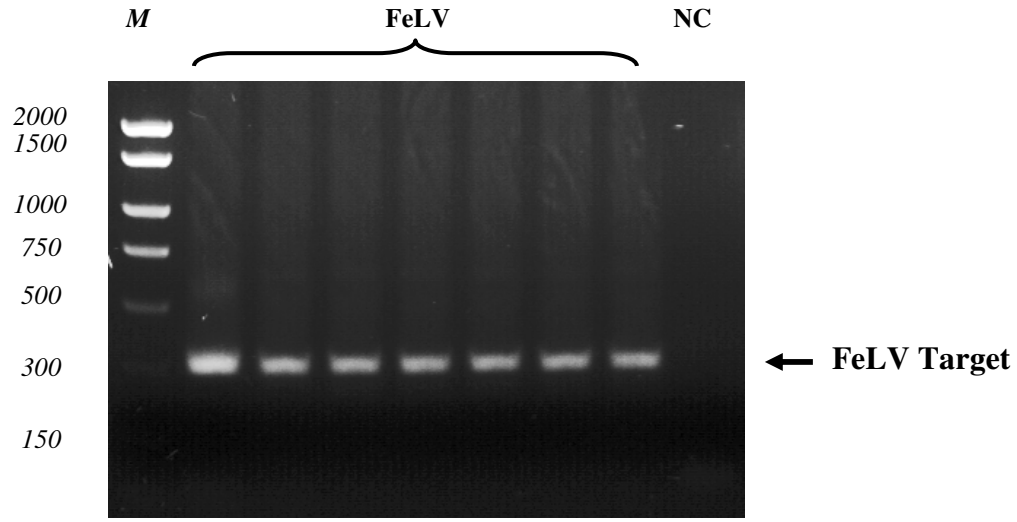

Figure 1: A representative 1X TAE 1.7% agarose gel showing the amplification of FeLV under different concentration (FeLV Target) using the **2X FeLV Detection RT-PCR Mastermix**. The size of the FeLV target amplicon corresponds to 310 bp as represented by the provided DNA Marker (M). **NC** = Negative Control.

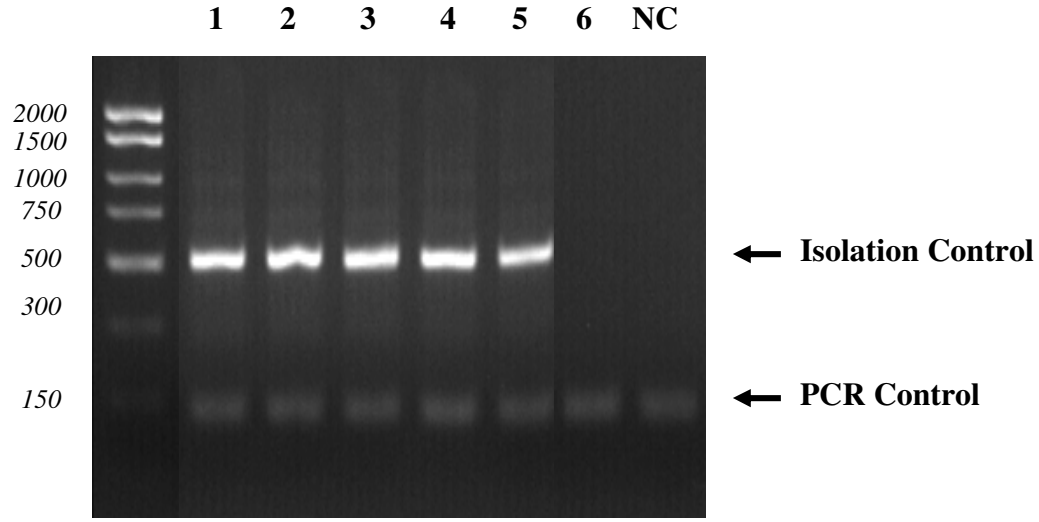

Figure 2: A representative 1X TAE 1.7% agarose gel showing the amplification of **Isolation Control** and **PCR Control** under different conditions using the **2X RT-PCR Control Mastermix**. The size of the Isolation Control amplicon and PCR Control amplicon correspond to 499 bp and 150 bp, respectively, as represented by the provided DNA Marker (M). Lanes 1 to 5 showed detection of both Isolation Control and PCR Control, suggesting that the RNA isolation as well as the RT-PCR reaction was successful. Lane 6 showed only the detection of PCR Control suggesting that while the RT-PCR was successful, the isolation failed to recover even the spiked-in Isolation control. **NC** = Negative Control.

Table 5. Interpretation of One-Step RT-PCR Assay Results

Input Type	Target reaction	Control Reaction		Interpretation
	FeLV Target Band (310 bp)	FeLV *IsoC* Band (499 bp)	FeLV *PCRC* Band (171 bp)	
Positive Control	X	X	X	Valid
Negative Control			X	Valid
Sample	X	X	X	Positive
Sample		X	X	Negative
Sample			X	Re-test
Sample				Re-test
Sample		X		Negative
Sample	X		X	Positive
Sample	X	X		Positive
Sample	X			Re-test

** For results obtained that are not covered in Table 5 above, please refer to the Troubleshooting Section.

E. FeLV RT-PCR Assay Specificity and Sensitivity

- The specificity of Norgen's FeLV RT-PCR Detection Kit is first and foremost ensured by the selection of the FeLV specific primers, as well as the selection of stringent reaction conditions. The FeLV specific primers were checked for possible homologies to all GenBank published sequences by sequence comparison analysis and published FeLV strains.

F. Linear Range

- The linear range of Norgen's FeLV RT-PCR Detection Kit was determined by analysing a dilution series of a FeLV quantification standards ranging from 100 ag to 1 pg.
- Each dilution has been tested in replicates (n = 4) using Norgen's FeLV RT-PCR Detection Kit on a 1X TAE 1.7% agarose gel.
- The linear range of Norgen's FeLV RT-PCR Detection Kit has been determined to cover concentrations from 100 ag to 1 ng
- Under the conditions of the Norgen's FeLV RNA Isolation procedure, Norgen's FeLV RT-PCR Detection Kit covers a linear range from 100 copies to 1 x 10^6 copies.

Frequently Asked Questions

1. How many samples should be included per RT-PCR run?

- Norgen's FeLV RT-PCR Detection Kit is designed to test 24 samples. For every 6 samples, a non-template control (Nuclease Free Water) and a Positive Control must be included. It is preferable to pool and test 6 samples at a time. If not, the provided Positive Control is enough to run 3 samples at a time.

2. How can I interpret my results if neither the FeLV RT-PCR control nor the FeLV Isolation Control (*IsoC*) amplifies?

- If neither the FeLV PCR control nor the FeLV Isolation Control (*IsoC*) amplifies, the sample must be re-tested. If the positive control showed amplification, then the problem occurred during the isolation, where as if the Positive control did not amplify, therefore the problem has occurred during the setup of the PCR assay reaction.

3. How should it be interpreted if only the FeLV PCR control showed amplification but neither the FeLV target nor the FeLV Isolation control amplified for a sample?

- This indicates a poor isolation. The isolation procedure must be repeated.

4. How should it be interpreted if only the FeLV Isolation Control (*IsoC*) was amplified in a sample?

- The sample tested can be considered as FeLV negative.

5. How should it be interpreted if the FeLV PCR control and the FeLV target showed amplification in a sample?

- The sample tested can be considered positive. It could happen when too much template was added to the reaction.

6. How should it be interpreted if only the FeLV target and the FeLV PCR control were amplified in a sample?

- The sample tested can be considered as FeLV positive.

7. How should it be interpreted if only the FeLV target was amplified in a sample?

- It is recommended that the isolation is repeated.

8. How should it be interpreted if only the FeLV PCR control and the FeLV Isolation control showed amplification in a sample?

- The sample tested can be considered negative

9. What if I forgot to do a dry spin after my third wash?

- Your first RNA elution will be contaminated with the Wash Solution. This may dilute the RNA yield in your first elution and it may interfere with the PCR detection, as ethanol is known to be a PCR inhibitor.

10. What if I forgot to add the FeLV Isolation Control (*IsoC*) during the isolation?

- It is recommended that the isolation is repeated.

11. What if I forgot to run the Control RT-PCR for the sample and I only ran the Detection RT-PCR and I obtained a positive result?

- The result can be considered positive. However, any negative result must be verified by running the associated control RT-PCR to ensure that it is a true negative and not a false negative due to problems with the RNA isolation or the RT-PCR reactions.

Related Products	Product #
Total RNA Purification Kit	17200
Sample Collection Kit For Upper Respiratory Tract Infectious Agents	29100
Feline Immunodeficiency Virus RT-PCR Detection Kit	44100
Feline Calicivirus RT-PCR Detection Kit	43900
Feline Herpes Virus PCR Detection Kit	44300
Feline *infectious peritonitis* RT-PCR Detection Kit	44400

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's FeLV RT-PCR Detection Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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