Result Analysis and Judgments

Result	Result Judgment
Ct value ≤ 36	Positive
36 < Ct value < 38	The sample need to be tested again, and the re-test results are still within this range or met positive criteria, and it is positive, otherwise it is negative.
No Ct value	Negative

Note

1. The whole process should be carried out in different area. Area I for preparing amplification reagent, area II for processing of tested samples, and area III for PCR amplification detection. All the articles in each district are for special use which cannot allow to be exchanged for avoiding pollution. The workbench should be cleaned immediately after the completion of each experiment.
2. Please handling samples in the biosafety cabinet, to ensure the safety of operators and avoid environmental pollution. Place harmful samples and reagents properly. Discard the waste in special containers. Wipe the table, centrifuge, and equipment frequently with 1.0% sodium hypochlorite or 70% ethanol. The laboratory and the ultra-clean workbench need UV-treated periodically and after each experiment.
3. Both the kit and nucleic acid products are all stored at -20℃. They should be fully melted, gently mixed and briefly centrifuged before use. Use disposable gloves without fluorescent substances, disposable special centrifuge tubes, etc.
4. After amplification, please take out the reaction tube, seal it in the special plastic bag, put it in the designated place, and wait for unified treatment.
5. The kit should be used within the shelf life. Reagents of different batches should not be used together.

Information of Manufacturer

Manufacturer: Hangzhou Bioer Technology Co., Ltd.
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PEOPLE'S REPUBLIC OF CHINA
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Feline leukemia virus Real Time RT-PCR Detection Kit

TECHNICAL SUPPORT:
For technical support, please dial phone number ： 0086-571-87774567-5287 or 5297,
or fax to 0086-571-87774553
Email to reagent@bioer.com.cn.
Website: www.bioer.com.cn

Intended Use

This kit is used for qualitative detection of *feline leukemia virus* in whole blood of feline animals. Feline leukemia is characterized by lymphoma, erythroblastic or myeloblastic leukemia, thymic atrophy, lymphocytopenia, neutropenia, and myeloid erythrocytic dysplasia anemia. It is a serious infectious disease of cats with high morbidity and mortality.

Principle

In this kit, *feline leukemia virus* specific gene was used to design primers and Taqman probes. The RNA was reverse transcribed into cDNA by reverse transcriptase, and combine with Taqman probe technology to achieve qualitative detection of *feline leukemia virus*.

Ingredients

Cat#	**BSL23S1**	**BSL23M1**
Components	**16 Tests**	**32 Tests**
RT-PCR Buffer	160 μL	320 μL
Enzyme Mix	16 μL	32 μL
FeLV Primer / Probe	112 μL	224 μL
FeLV Positive Control	64 μL	64 μL
FeLV Negative Control	64 μL	64 μL

Storage and period of validity

1. The kit need to be transported under freezing conditions.
2. The kit should be stored at -15℃ ~ -25℃ away from light, and avoid repeated freeze-thaw more than 5 times.
3. The kit can be stored for up to 12 months if all components are kept in the manner above. Please use the kit in the period of validity.

Applied instruments

It can be used Line-Gene and Quant-Gene 9600 series fluorescence quantitative PCR detection system from Bioer or similar instruments from other manufacturers.

Sample request

1. The samples should be placed in sterile and clean tubes to avoid cross -contaminations; if not immediately extract, the samples should be stored at -20 ℃ or -80 ℃.
2. The DNA detected by the kit should be extracted from the Nasopharyngeal swabs, feces, serum and lung, lymph node and other tissue samples of cats.
3. If the extracted DNA is not used immediately, it should be stored at -20 ℃ or -80℃.

Using of the kit

1. Nucleic acid extraction

Samples can be extracted using the SimplyP Animal pathogens DNA/RNA Extraction Kit (Cat No.: BSC70T1、BSC70S1、BSC70M1), and MagaBio plus Virus DNA/RNA Purification Kit II (Cat No.: BSC71S1C、BSC71S1E) from Bioer.

2. Amplification reagent preparation

Thaw out the reagents at room temperature. Before preparing PCR reagents, mix gently and centrifuge briefly all reagents. Make PCR reagents according to the quantity of samples and controls as below (N tests add an extra blank control):

Reagent	RT-PCR Buffer	Enzyme Mix	FeLV Primer / Probe
Dosage / test	10 μL	1 μL	7 μL
Dosage	(N+1) ×10 μL	(N+1) ×1 μL	(N+1) × 7 μL

After mixing PCR reagents above, distribute 18 μL into every PCR tubes, and then transfer to sample disposal area. Add 2 μL extracted sample, positive control and negative control into every PCR tube. Tighten the tube cover, remove bubbles by centrifugation, and then conduct PCR reaction.

3. PCR reaction

Set reaction procedure as following:

50℃ 10 min
95℃ 1 min
95℃ 10 sec
60℃ 20 sec
} 40 Cycles

Select the FAM channel of instrument for fluorescent signal collection. Instrument set fluorescent signals detecting at 60℃, reagent volume is 20 μL.

Quality control standards

	Result	**Interpretation of Test Results**
Positive Control	Ct value ≤ 30	All conditions are met in the same experiment, indicating that the experiment is valid, otherwise it is invalid.
Negative Control	No Ct value	