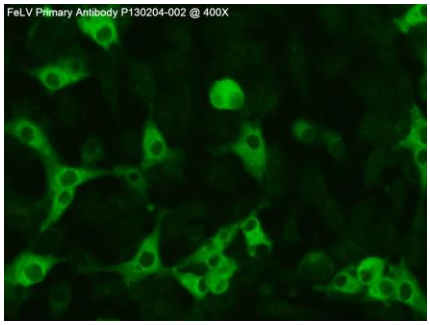
Veterinary Medical Research & Development

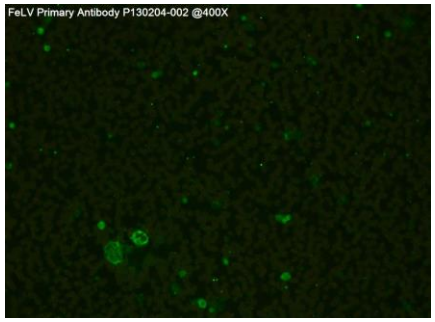
CERTIFICATE OF ANALYSIS

PRIMARY ANTI-FELINE LEUKEMIA VIRUS (FeLV)

Antiserum for IFA

Catalog No.:	AB1-FELV
Volume:	10 ml
Lot:	P130204-002
Expiration:	14 May 2017
Agent:	Feline Leukemia Virus (FeLV)
Strain:	NA





Description:

FeLV polyclonal antiserum. Goat origin. Useful for detection of Feline Leukemia Virus by IFA (procedure follows). FeLV antiserum specifically binds FeLV antigens in peripheral blood leukocytes, necropsy tissues, or tissue culture.

Quality Control Method:

IFA using FeLV 2-well slide (catalog no. SLD-FAC-FELV), FeLV positive blood smear (catalog no. SLD-BSP-FELV), FeLV negative blood smear (catalog no. SLD-BSN-FELV), and FeLV secondary reagent (catalog no. AB2-FELV).

Specific Reaction: 2-4+ fluorescence with trace to 1+ background on the positive well and negative with trace to 1+ background on the negative well. 3-4+ fluorescence, no background on the positive blood smear and negative, no background on the negative blood smear.

Other Comments: NA

Pattern Of Fluorescence:

On the 2 well slide (SLD-FAC-FELV) there should be Individual cell cytoplasmic fluorescence with occasional small syncytia. On the Positive Blood Smear there is membrane and cytoplasmic fluorescence on platelets and white blood cells.

Intended Use:

Primary antibody is useful for detection of Feline Leukemia Virus by IFA (procedure follows).

Storage:

This antiserum is provided in liquid form and should be stored at 2-7°C. DO NOT FREEZE! If antiserum becomes cloudy, it should be discarded. This antiserum contains 0.09% sodium azide as a preservative.

References: NA

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Detection of Feline Leukemia Virus by Indirect Fluorescent Antibody

I. PERIPHERAL BLOOD LEUKOCYTES

1. Buffy coat smears are preferred, but thin, feathered-edge blood smears (as made for a WBC differential) may be used for this test. They should be air dried, but not over one week old. Unstained smears should be stored at room temperature in a dark, dry place and should NOT be refrigerated due to the fact that any moisture condensing on the surface of the slide will ruin it for staining. Unstained smears may be preserved long term by storing in acetone-methanol (75/25%) at Revco temperatures (-76°C to -90°C). Fresh blood in anticoagulant should be mixed very thoroughly before smearing on clean, new slides. Fresh slides should be dried at least 30 minutes at room temperature or 15 minutes at 37°C before fixing.
2. Slides should be fixed for 20 minutes at room temperature in a mixture of 75% acetone and 25% methanol. This fixative may be mixed up in bulk in advance and stored at room temperature in a tightly capped vessel. Allow the slides to air dry after fixing and then circle (10-15 mm) the feathered edge with a Mark-Tex pen, liquid embroidery, grease pencil, or other suitable means.
3. Place 50-100 µl (1-2 drops) of Anti-FeLV Antiserum (VMRD catalog no. AB1-FELV), white label, primary antibody) in the circle and gently spread to cover. Incubate slides 30 minutes at 37°C in a humid chamber.
4. At the end of the primary incubation period, rinse the slide gently for 5-10 seconds in FA Rinse Buffer (VMRD catalog no. FARB-4X) and then soak in FA rinse buffer for 10 minutes. Drain the slide and dry around the circle with a Kleenex or paper towel. Do not allow the smear inside the circle to dry.
5. Place 1-2 drops of Anti-IgG FITC Conjugate (VMRD catalog no. AB2-FELV, yellow label, secondary fluoresceinated antibody) in the circle and incubate 30 minutes at 37°C in a humid chamber.
6. Rinse as before for 10 minutes and drain. Dry the *back* of the slide (do not allow the front to dry).
7. Place one drop of mounting medium (50% FA rinse buffer/50% glycerol; VMRD catalog no. FAMF-10ML) on the circle, mount with a coverslip and read on a good quality fluorescence microscope. Scan at 100-250X and confirm at 400X.
8. Stained RBC's will appear red to orange/brown. FeLV negative leukocytes will not be visible except for eosinophils, which will be white to pale yellow. Positive cells will appear bright apple green: neutrophils, eosinophils, lymphocytes, platelets and/or large lymphoblasts. The cells may be concentrated at the edge of the feathered area and large clumps of positive platelets or lymphocytes may be seen.

II. POST MORTEM TISSUES:

1. Impression smears of the cut surface of spleen, thymus, or lymph node may be made and processed as for blood smears. Frozen sections of any tissue may also be used and stained as above.
2. Bone marrow aspirates may be stained either from live cats or from post-mortem sources. Impressions or sections of biopsied tumors may be stained as an aid in the identification of FeLV-induced lymphosarcomas.

III. TISSUE CULTURES:

1. FeLV infection in tissue cultures may also be confirmed by this method. The protocol in Section I should be used, with the substitution of pure (100%) acetone fixation for 10 minutes instead of 75% acetone and 25% methanol as in Step 2 of Section I.
2. This protocol may be used in performing serum neutralization tests on cat sera by staining the culture 5-7 days after planting.

4X FA RINSE BUFFER (pH 9.0):

- Na_2CO_3 . . . . . 11.4 gm
- $NaHCO_3$ . . . . . 33.6 gm
- NaCl . . . . . 8.5 gm
- DI/dH_2O . . . . . Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1/4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.