INSTRUCTIONS FOR USE

Feline Immunodeficiency Virus (FIV)
IFA Antibody Kit

Catalog Number:	FIV-120
Size:	120 test
Storage:	2-8˚C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against Feline Immunodeficiency Virus (FIV) in feline serum or plasma

For in-vitro diagnostic use only

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INTENDED USE

The Feline Immunodeficiency Virus IFA Antibody kit is intended for the detection of feline antibody to FIV antigens.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed feline kidney cells (Crandall), approximately 7-15% of which are infected with Feline Immunodeficiency Virus and contain the characteristic cytoplasmic fluorescence. Feline sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of serum antibody with the fixed antigens. Slides are then washed to remove non-reactive serum proteins, and fluorescence-labeled anti-feline IgG (conjugate) is added. This conjugate is allowed time to react with antigen-antibody complexes. The slides are washed again to remove non-reactive conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as fairly homogenous apple-green cytoplasmic fluorescence in 7-15% of the cells in each field. A negative reaction is seen either as red-counterstained cells or fluorescence unlike that seen in the positive control well.

REAGENTS

IFA | Ag x 12 **Substrate Slides (10)**
10x12-well masked slides containing uninfected and FIV-infected cells. Slides are pre-fixed and ready to use.

CONJ | FITC **Conjugate, 2.5 mL**
Yellow cap dropper bottle contains affinity-purified AlexaFluor-labeled goat anti-feline IgG (heavy chain) with bovine serum albumin and Evans' blue counterstain.

CONT | + **Positive Control, 0.5 mL**
Blue cap dropper bottle contains reactive feline serum, provided at a 1:10 screening dilution. Endpoint titer is 1:80.

CONT | – **Negative Control, 0.5 mL**
Red cap dropper bottle contains non-reactive feline serum, provided at a 1:10 screening dilution.

MM **Mounting Medium, 1 mL**
White cap dropper bottle contains glycerol (50% v/v) in PBS

BUF | WASH | PBS **PBS, 1 liter**
Add supplied powder to 1 liter purified water to produce PBS.

Warnings
Since no testing can assure the absence of infectious agents, these reagents, as well as all serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion. The substrate slides are prepared with chemically inactivated antigens, although they should be considered potentially infectious and handled accordingly.

Storage and Handling
Kit components should be stored at 2-8°C. Bring them to room temperature (20°-25°C) before opening bottles or slide envelops.

SPECIMEN COLLECTION

Allow blood samples to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8°C. If testing is to be delayed longer than 5 days, freezing the sample at -20°C or colder is recommended.

PROCEDURE

The kit supplies sufficient reagents and materials for 120 determinations.

Materials Required But Not Supplied

- Distilled or deionized water
- Clean 250 or 500 mL wash bottle for PBS
- Test tubes or microtiter plate for serum dilutions
- Precision pipette(s)
- 24 x 50 mm glass coverslips
- Fluorescence microscope with filter system for FITC (maximum excitation wavelength 490 nm, mean emission wavelength 530 nm) and 400X magnification.
- 37° waterbath or incubator
- Humid chamber for slide incubation steps.

Precautions

- Do not use components past expiration date.
- Conjugate is photosensitive and is packaged in opaque plastic for protection. Store in the dark and return to storage after use.
- Conjugate contains Evans' blue dye, which may be carcinogenic. Avoid contact with skin.
- Liquid reagents contain thimerosal at 0.001%, which may be toxic if ingested

ASSAY PROCEDURE

1. Prepare 1:10 screening dilutions for all untested sera.

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:40-1:160).

3. For each serum dilution add 15 µL to one slide well and record the location for later reference. For each assay run include the Negative Control and dilutions of the Positive Control prepared above.

4. Place slides in a humid chamber and incubate for 30 minutes at 37°± 0.5°C.

5. Remove humid chamber from incubator. Rinse slide wells with gentle stream of PBS from wash bottle. Shake or tap beaded PBS from slides into a sink, then repeat this wash step 3X without allowing the wells to dry.

6. To each slide well add 1 drop (15-20 µL) conjugate and return slides to the humid chamber for another 30 minutes incubation at 37°± 0.5°C. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slides as in step 5, above.

8. Add 3-4 drops of Mounting Medium to each slide and apply coverglass.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the cytoplasmic fluorescence seen in the Positive and Negative Control wells. Slides may be stored at 2-8°C in the dark for up to 24 hours.

QUALITY CONTROL

The Negative Control serum and dilutions of the Positive Control serum should be assayed with each daily run. The Negative Control well is an example of a non-reactive serum, with either uniform red counter stain or slight, but uniform greenish staining across all of the cell (both infected and uninfected). The Positive Control wells should give an endpoint titer from 1:40 to 1:160. The fluorescence intensity at 1:80 may be used as the cut-off level required for a serum reaction to be called positive. If either of the Controls does not react as specified, the assay run should considered void, reagent components and procedural steps rechecked, and the assay repeated from step #1.

INTERPRETATION OF RESULTS

A positive reaction appears as fairly homogenous cytoplasmic fluorescence only in infected cells. The size, appearance and density of the fluorescence must be compared with the Positive and Negative Control reactions. Patterns of reactivity different than that seen in the Positive Control must be considered non-specific.

Patient Specimens

Positive at 1:10 screening dilution: IgG titers of 1:10 and greater are considered to reflect infection, unless the cat has been vaccinated for FIV.

Negative at 1:10: Report as negative for FIV antibody.

LIMITATIONS

This test cannot distinguish between a wild type infection and a post-vaccination response.

New 12/2011
Initial Version