INSTRUCTIONS FOR USE

Feline Infectious Peritonitis Virus
IFA Antibody Kit

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

An Indirect fluorescence immunoassay for the detection of IgG class antibody against Feline Infectious Peritonitis Virus in feline serum or plasma

For in-vitro diagnostic use only

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INTENDED USE
The Feline Infectious Peritonitis Virus IFA Antibody kit is intended for the detection and semi-quantitation of feline antibody to Feline Infectious Peritonitis Virus.

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 Infectious Peritonitis (FIP) Virus and contain the characteristic granular cytoplasmic fluorescence. Feline sera are diluted in buffered saline and incubated in the individual slide wells to allow reaction of antibody with the fixed antigens. Slides are then washed to remove unreacted 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 unreacted Conjugate. The resulting reactions can be visualized using standard fluorescence microscopy, where a positive reaction is seen as granular apple-green fluorescence within the cytoplasm of 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. Positive reactions may then be retested at higher dilutions to determine the highest reactive or endpoint dilution.

REAGENTS

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

CONJ | FITC **Conjugate, 2.5 mL**
Yellow cap dropper bottle contains affinity-purified DyLight 488-labeled goat anti-feline IgG (heavy and light chains) with bovine serum albumin and Evans' blue counterstain.

CONT | + **Positive Control, 0.5 mL**
Blue cap dropper bottle contains reactive feline serum at a 1:20 screening dilution. Endpoint titer is 1:160

CONT | – **Negative Control, 0.5 mL**
Red cap dropper bottle contains non-reactive feline serum at a 1:20 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
The control sera have been screened for infectious agents by USFDA required testing. Since no testing can assure the absence of infectious agents, however, 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. However, the slides should be considered potentially infectious and handled accordingly.

Storage
Kit components should be stored at 2-8ºC or colder. Bring them to room temperature (20-25ºC) before opening bottles or slide envelopes.

SPECIMEN COLLECTION
Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. Store at 2-8ºC. If testing is to be delayed longer than 5 days, freezing the sample at -20ºC or colder is recommended. Acute specimens are drawn at the onset of illness and convalescent specimens obtained at intervals to check for titer changes.

PROCEDURE
The kit supplies sufficient reagents and materials for 120 determinations.

Materials Required But Not Supplied
- Purified (distilled or deionized) water
- Clean 250 or 500 mL wash bottle for PBS
- Wash bath with slide rack
- Test tubes or microtiter plate for diluting
- Precision pipette (15 µL)
- 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
- 37ºC water bath or incubator
- Humidity chamber for slide incubation steps

Precautions
- Do not use components past expiration date.
- Conjugate is photosensitive
- 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

Allow all reagents and sera to reach ambient temperature before starting timed assay procedure.

1. Prepare 1:20 screening dilutions in PBS for patient serum specimens. For sera found positive on a previous assay run, prepare serial dilutions in PBS, starting with screening dilution (as above).

2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:80-1:320).

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 dilutions of the Positive Control prepared in step 2 and 1 drop of the Negative Control to one well.

4. Place slides into a humidity chamber and incubate for 30 minutes at 37º± 0.5ºC.

5. Rinse slide wells with gentle stream of PBS from wash bottle three (3) times. Shake or tap excess PBS from slides held with beads of PBS and go directly to next step without allowing slide wells to dry

6. To each slide well add 1 drop of Conjugate, then return slides to humidity chamber for 30 minute incubation at 37º± 0.5ºC. Incubation should be in the dark to protect the photosensitive conjugate.

7. Wash slides as in step 5, above.

8. Add 2-3 drops of Mounting Medium to each slide and apply cover glass.

9. Read the stained substrate slides at 400X magnification, comparing each well to the visual intensity and appearance of the Positive and Negative Control wells. Slides may be stored at 2-8ºC 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 counterstain or slight, but uniform greenish staining. The Positive Control wells should give an endpoint titer from 1:80 to 1:320. The fluorescence intensity at 1:160 may be used as the cut-off level required for a reaction to be called positive. If either of the Controls does not react as specified, the assay run should be considered void, reagent components and procedural steps should be rechecked, and the assay repeated from the beginning.

INTERPRETATION OF RESULTS
A positive reaction appears as bright granular fluorescence seen in the cytoplasm of infected cells. The size, appearance and density of the inclusions 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 1:20 screening dilution: IgG titers of 1:20 and greater are considered to reflect infection by Feline Coronavirus at an undetermined time **(please see Limitations section).** Sera positive at the 1:20 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same cat.

Negative at 1:20: Report as negative for FIP antibody.

LIMITATIONS
FIP is caused by Feline Coronavirus (FCV), a commonly encountered virus One way to proceed is to make sure that the patient has at least two of the following clinical signs:
1) fever of unknown origin
2) white blood cell count over 20,000 with high percentage of neutrophils (90%)
3) Total serum protein over 8 g/dL
4) Abdominal or thoracic fluid with over 20,000 leukocytes/µL
5) Severe uveitis

If patient is demonstrating two or more of these signs and has an IFA antibody titer of 1:2,000 or above, the chances are very good that FIP is the clinical diagnosis. Lower antibody titers (1:400-1:2,000) could also indicate FIP if clinical signs are present.

EXPECTED VALUES
The prevalence of FIP antibodies varies depending upon the geographic region and population being tested. Low titers (<1:2,000) are relatively common (see Limitations, above).

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