

INSTRUCTIONS FOR USE

Canine Distemper IFA Antibody Kit

Catalog Number: CDG-120

Size: 120 test

Storage: 2-8°C

An Indirect fluorescence immunoassay for the detection of IgG class antibody against Canine Distemper Virus in canine serum or plasma

For in-vitro diagnostic use only



1135 E. Truslow Avenue
Fullerton, California 92831 USA
Phone: +1-714-525-7660
Fax +1-714-525-7614
Email: info@fullerlabs.com
URL: www.fullerlabs.com



MediMark Europe Sarl
11, rue Émile Zola – BP 2332
F-38033 Grenoble Cedex 2 – France

INTENDED USE

The Canine Distemper IFA Antibody Kit is intended for the detection and semi-quantitation of canine antibody to Canine Distemper Virus.

SUMMARY AND EXPLANATION OF TEST

Substrate slides consist of teflon-masked wells containing fixed mink lung fibroblasts, approximately 7-15% of which are infected with Canine Distemper Virus and contain the characteristic cytoplasmic inclusion bodies. Canine 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 unreacted serum proteins before DyLight 488-labeled anti-canine IgG (conjugate) is added. After this conjugate has reacted with the 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 sharply defined apple-green fluorescent inclusions within the cytoplasm of 7-15% of the cells in each field. A negative reaction is seen either as red-counterstained cells or as 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 CDV-infected 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 rabbit anti-canine IgG (heavy and light chain) with bovine serum albumin and Evans' blue counterstain.

CONT +

Positive Control, 0.5 mL

Blue cap dropper bottle contains reactive canine serum, provided at a 1:25 screening dilution. Endpoint titer is 1:200.

CONT -

Negative Control, 0.5 mL

Red cap dropper bottle contains non-reactive canine serum, provided at a 1:25 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. However, the slides 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 envelopes.

SPECIMENS

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing sterile containers. 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. Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained at two and four week intervals to check for titer changes.

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.
- 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 1:25 screening dilutions for all untested sera. For sera found positive on a previous assay run, prepare serial two-fold dilutions in PBS, starting with 1:25.
2. Prepare dilutions of the Positive Control to include 1 dilution above the stated endpoint and one dilution below (ie. 1:100-1:400).
3. For each serum dilution add 15 µL to one slide well. For each assay 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. Also remove conjugate from storage. Rinse slide wells with gentle stream of PBS from washbottle. 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, then return slides to the humid chamber for 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 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 viral inclusions 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 counterstain or slight, but uniform greenish staining. The Positive Control wells should give an endpoint titer from 1:100 to 1:400. The fluorescence intensity at 1:200 may be used as the cut-off level required for a patient 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 rechecked, and the assay repeated from step #1.

INTERPRETATION OF RESULTS

A positive reaction appears as bright inclusion bodies 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 1:25 screening dilution: IgG titers of 1:25 and greater are considered to reflect infection at an undetermined time. Sera positive at the 1:25 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same dog.

Negative at 1:25: Report as negative for Canine Distemper antibody.

LIMITATIONS

IgG class antibody induced by Distemper vaccination or past infection is a limitation in assessing antibody levels. Post-vaccination levels may remain elevated for long periods. Also IgG antibody levels may be elevated on presentation and no longer increasing at presentation. CNS infection can be accurately diagnosed by elevated CSF titers, although damage to the blood-brain barrier must be taken into account and the lack of a titer does not rule out CDV.

In the absence of vaccination within a 3-week period, a positive IgM titer can also provide an accurate diagnosis for up to 3 months post onset. This assay requires serum pretreatment for removal of rheumatoid factor and excess specific IgG as adverse assay factors.

EXPECTED VALUES

The prevalence of Canine Distemper Virus antibodies varies depending upon the geographic region and population being tested. Unexposed dogs should be non-reactive at 1:25.

New 12/2000

Initial Version

Revised 12/2009