

***LEISHMANIA INFANTUM* Antigen**

INSTRUCTION LEAFLET

1ml vial of 5 .10⁶ parasite suspension /ml

COMPONENT

Formol-inactivated suspension of *Leishmania infantum* promastigote parasites, used for anti-*Leishmania* specific antibodies titration by Indirect Immunofluorescence test.

INDICATION OF USE

Immunological revelation and titration of anti-*Leishmania infantum* antibodies in canine serum, mainly with the Indirect Immunofluorescence technique (IIF).

PROCEDURE : Anti-*Leishmania infantum* antibodies titration by IIF

Required material (not supplied):

- 18 well slides and small slides
- 96 well microtiter plates
- Multichannel pipettors to measure and deliver 100 and 25 μ l
- Micronic or similar tubes
- Hair drier
- UV microscope (X40 or higher magnification)
- Wet incubator at +37°C +/-3°C
- Fluorescein-labelled conjugate (Anti-canine Ig antiserum or Protein A)
(Dilution of use should be previously selected by testing control sera)
- PBS without calcium and magnesium
- Cold acetone
- 1% Evans blue solution
- Fluoprep

Preparation of slides:

- Place 10 μ l of the parasite suspension per well
- Dry slides with an overnight incubation at room temperature (+20°C +/-5°C)
- Dip slides 15 min. in cold acetone (-20°C) for fixation
- Store ready-to-use slides at -20°C (up to 6 months)

Immunofluorescence test:

- Prepare a 1:12.5 predilution for each serum under test in PBS without calcium and magnesium (in micronic or similar tubes)
- Prepare a set of seven serial two fold dilutions in a 96 wells microtiter plate:

25_____50_____100_____200_____400_____800_____1600

- Extend to higher dilutions for high titered samples
- Place 20µl of each dilution per well
- Incubate for 30 min. +/- 5min. at +37°C +/-3°C in a wet incubator
- Wash slides twice by 5 min.diping in PBS without calcium and magnesium
- Rinse slides with distilled water and dry with hair drier
- Place 20µl of the appropriate conjugate dilution in PBS without calcium and magnesium supplemented at 1:1000 with the 1% Evans blue solution
- Incubate for 30 min. +/- 5 min. at +37°C +/-3°C in a wet incubator
- Wash slides twice by 5 min.diping in PBS without calcium and magnesium
- Rinse slides with distilled water and dry with hair drier
- Set the slides with fluoprep
- Read the fluorescence on UV microscope
- The serum titer is expressed as the reciprocal of the highest dilution providing a positive fluorescence

STORAGE

Store parasite suspension at + 5°C +/- 3°C until expiry date.

PRESENTATION

1ml vial.

FOR VETERINARY USE ONLY / FOR IN VITRO USE ONLY
SYNBIOTICS EUROPE - 2 rue Alexander Fleming - 69367 LYON Cedex 07 – France
Tel : +33 4.72.76.11.11 - Fax : +33 4.72.76.11.10
www.synbiotics.fr info@synbiotics.fr