

## **INSTRUCTION LEAFLET**

### **1ml vial**

### **COMPONENT**

Formol-inactivated suspension of cells ( $2.10^6$  cells/ml) infected with the bacteria Ehrlichia canis, used for anti-E. canis specific antibodies titration by Indirect ImmunoFluorescence test.

### **INDICATION OF USE**

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

### **PROCEDURE : Anti-E. canis 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  $\mu$ l
- Micronic or similar tubes
- Hair drier
- UV microscope ( X40 or higher magnification)
- Wet incubator at  $+37^{\circ}\text{C} \pm 3^{\circ}\text{C}$
- 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
- Bovine Serum Albumin (BSA)
- Cold acetone
- Fluoprep

#### **Preparation of slides:**

- Adjust the cellular suspension at  $10^6$  cells/ml by 1:2 dilution in PBS without calcium and magnesium but supplemented with 0.5% BSA
- Place 10 $\mu$ l of the cellular suspension per well
- Dry slides with an overnight incubation at room temperature ( $+20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ )
- Dip slides 15 min. in cold acetone ( $-20^{\circ}\text{C}$ ) for fixation
- Store ready-to-use slides at  $-20^{\circ}\text{C}$  (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.dipping 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
- Incubate for 30 min. +/- 5min. at +37°C +/-3°C in a wet incubator
- Wash slides twice by 5 min.dipping 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 cell suspension at + 5°C +/- 3°C until expiry date.

### **PRESENTATION**

1ml vial.

FOR VETERINARY USE ONLY / FOR IN VITRO USE ONLY  
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