ProtaTek International, Inc.

Indirect Fluorescent Antibody (IFA) Testing

Indirect Fluorescent Antibody Slides Available

Organism	Disease	Price
Rickettsial:		
♦*Ehrlichia canis*	Canine Ehrlichiosis	1-49 $27 ea 50-99 $25 ea 100-199 $23 ea 200 + $20 ea
♦*Ehrlichia equi (Anaplasma Phagocytophila)*	Equine Ehrlichiosis	1-49 $28 ea 50-99 $26 ea 100-199 $24 ea 200 + $22 ea
♦*Ehrlichia risticii*	Equine Monocytic Ehrlichiosis (Potomac Horse Fever)	1-49 $20 ea 50-99 $18 ea 100-199 $15 ea 200 + $ 12 ea
♦*Rickettsia rickettsii* **While supplies last**	Rocky Mountain Spotted Fever	1-49 $17 ea 50-99 $14 ea 100-199 $12 ea 200 + $ 10 ea
Viral		
♦Canine Distemper virus	Canine Distemper	Not Making At This Time
♦Canine Parvovirus	Canine Parvovirus Infection	Not Making At This Time
Bacterial		
♦*Borrelia burgdorferi*	Borreliosis- Lyme Disease	$10.00 ea
Protozoan		
♦ *Babesia canis*	Canine Babesiosis	1-49 $24 ea 50-99 $22 ea 100-199 $20 ea 200 + $18 ea
♦*Babesia Gibsoni*	Canine Babesiosis	1-50 $30 ea 50-100 $28 ea 100-199 $26 ea 200 + $24 ea

We also have Toxoplasma Gondii Slides that are $9.00 ea.

Positive and Negative Control Serum is also Available.
Positive is $12.00ea and Negative is $5.00.
All International Orders are Pre-Pay Only. And there is an additional $30.00 service charge. Any questions please contact ProtaTek.

ProtaTek International, Inc.
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ProtaTek Research Procedures For IFA Analysis

www.protatek.com

Principle of the Test:

The test is designed to detect antibodies of the immunoglobulin G (IgG) class in serum or plasma obtained either from actively infected animals or vaccinates. The later animals may or may not have been challenged with the live virulent organism following vaccination prior to collecting their serum or plasma.

Basic Procedures:

A. Incubation with Serum or Plasma (Primary Antibody)

1. Prepare the positive and negative control dilutions:
   a. Positive Control- 1:640
   b. Negative Control- 1:40
2. Spot the wells with 20-25µl of the appropriate serum controls
3. Incubate the slides at 37°C, in the dark, for 20-30 minutes
4. Knock off the serum controls, being careful not to contaminate the negative with positive serum
5. Wash the slides, while vigorously mixing on a rotary shaker, two times in potassium phosphate buffer (pH 7.2) with 0.75% Tween (PBST) for five minutes, followed by two washes in distilled water for two and a half minutes
6. Dry the slides completely

B. Incubation with Conjugate (FITC-conjugated secondary antibody)

1. Prepare the conjugate dilution 1:500
2. Spot each well with 20-25µl of conjugate
3. Incubate the slides at 37°C, in the dark, for 20-30 minutes
4. Knock off the conjugate
5. Repeat the washes as previously described

6. Dry slides completely

C. Microscopic Analysis

1. Add 10-15µl of 90% glycerol/ 10% PBS to each well
2. Place a cover slip on the slide
3. Observe the slide using the UV light microscope

Interpretation of Results:

The positive serum/ plasma must show parasite-specific, bright green-yellow fluorescence. The negative serum/ plasma must show no parasite-specific fluorescence at all. Background or non-specific fluorescence is quenched using Evans Blue dye as a counter stain incorporated into the conjugate at a dilution of 1:8000 (*Ehrlichia risticii*) or 1:500 (*Ehrlichia canis*) of a 0.5% stock solution. The counter stain imparts varying degrees of rusty-brown or dark red staining of the host cell.

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