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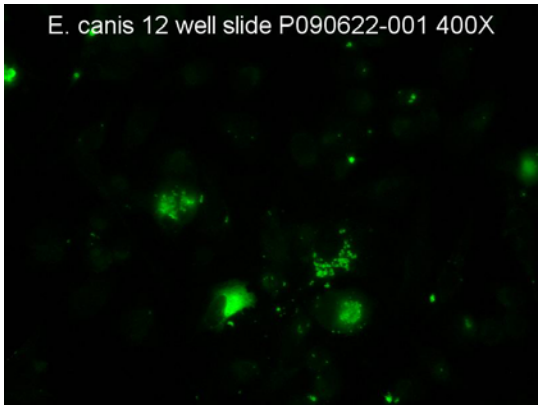
Certificate of Analysis

CANINE EHRLICHIOSIS

FA Substrate Slide



CATALOG NO.: SLD-IFA-EC
SIZE: 12 Well
LOT: P090622-001
EXPIRATION: 28 July 2011

AGENT: *Ehrlichia canis*
ISOLATE: CDC/V241
CELL CULTURE SUBSTRATE: DH82 cells

DESCRIPTION: Slides contain fixed *E. canis* in DH82 cells (licensed under U.S. Patent No. 5,192,679). Cells are unstained and slides are sealed in moisture-free foil pouches.

QUALITY CONTROL METHOD: IFA using VMRD, Inc. *Ehrlichia canis* Positive Control (catalog no. 211-P-EC), *Ehrlichia canis* Negative Control (catalog no. 211-N-EC), and Anti-Canine IgG FITC Conjugate (catalog no. 035-1 or 035-10).

Specific Reaction: 4+ signal with positive control, trace background with negative control.
Other Comments: 0-25 infected cells per high powered field.

PATTERN OF FLUORESCENCE: Brightly fluorescent cytoplasmic inclusion bodies (morulae), clustered elementary bodies (initial bodies), and free elementary bodies, approximately 0.3 microns in diameter.

INTERPRETATION OF RESULTS: Titers of 1:50 (IgG) and greater are considered positive. Positive control sera, with stated endpoints, are available on request (catalog no. 211-P-EC).

INTENDED USE: Generally used for Indirect FA to detect antibody to *E. canis* but may also be used as a positive and negative control substrate slide for Direct FA conjugates when applicable.

STORAGE: Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

REFERENCES: NA

FOR *IN VITRO* LABORATORY USE ONLY.

RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 50 µl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. 210-93-SB).
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. 210-90-RB) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry around wells by pressing blotter (included in pouch) to front surface. Place 50 µl labeled anti-IgG or IgM on the wells.
6. Incubate as in step 3.
7. Rinse as in step 4.
8. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
9. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. 210-92-MF) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be made at 400X.

RECOMMENDED STAINING PROCEDURE FOR DIRECT FA:

1. Warm slide to room temperature before removing from foil pouch.
2. Place 50 µl of direct FA conjugate on the designated wells.
3. Incubate slide in humid chamber at 37°C for 30 minutes.
4. Using a wash bottle, gently rinse slide briefly in FA rinse buffer, pH 9.0 (catalog no. 210-90-RB) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
5. Drain slide and dry back and edges with a paper towel. Do not allow stained surface to dry. Do not rinse with water.
6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. 210-92-MF) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

*SERUM DILUTING BUFFER (pH 7.2):**

- Na_2HPO_4 . . . . . 1.19 gm
- NaH_2PO_4 . . . . . 0.22 gm
- NaCl . . . . . 8.55 gm
- BSA . . . . . 10.0 gm
- DI/dH_2O . . . . . Q.S. to 1 liter

* This recipe makes 1 liter. If you need less, adjust recipe accordingly. Store at 2-7°C. Add 0.09% NaN_3 if diluted serum is not going to be used within one week.

4X FA RINSE BUFFER (pH 9.0):

- Na_2CO_3 . . . . . 11.4 gm
- $NaHCO_3$ . . . . . 33.6 gm
- NaCl . . . . . 8.5 gm
- DI/dH_2O . . . . . Q.S. to 1 liter

Final pH should be 9.0-9.5. This is a 4X concentrate and should be diluted 1:4 with DI/distilled water for use as a working buffer. Keep in a tightly stoppered container at room temperature. MOUNTING FLUID is made by mixing glycerol and FA rinse buffer, pH 9.0, in equal proportions.