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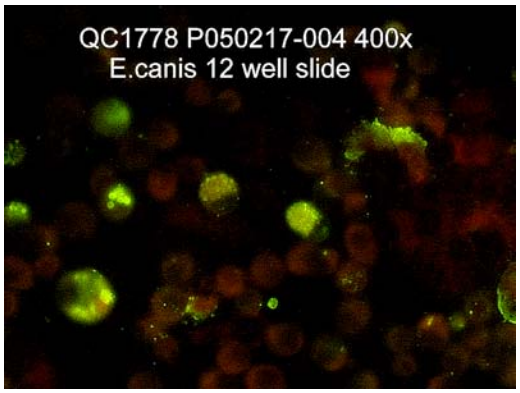
Certificate of Analysis

CANINE EHRLICHIOSIS

FA Substrate Slide

CATALOG NO.: 210-88-12-EC
SIZE: 12 well
LOT: P050217-004-040707
EXPIRATION DATE: 07 April 2007



AGENT: *Ehrlichia canis*
Cell Culture Substrate: DH82 cells
Strain: Oklahoma (LSU)

QUALITY CONTROL METHOD: Indirect FA using VMRD *Ehrlichia canis* Positive Control (catalog no. 211-P-EC), *Ehrlichia canis* Negative Control (catalog no. 211-N-EC), Anti-Canine IgG FITC Conjugate (catalog no. 035-10).
Specific Reaction: 3-4+ positive on positive cells.
Other Reactions or Comments: 10-30 positive cells per high power 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.

INTENDED USE: For detection of antibody to *E. canis* by indirect FA technique. May be used to differentiate antibody class (IgG or IgM) with suitable quality fluoresceinated second antibody conjugate.

STABILITY: Foil-pouch sealed slides are stable for at least 6 months when stored below 10°C. Avoid self-defrosting freezers.

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

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).

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 10 µl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. 210-93-SB). [If background is a problem, particularly at low dilutions, use of 10% adult bovine serum diluting buffer is preferable (catalog no. 210-94-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 flick to remove excess moisture. Place 10 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 <u>back and edges</u> 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.

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 4°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.