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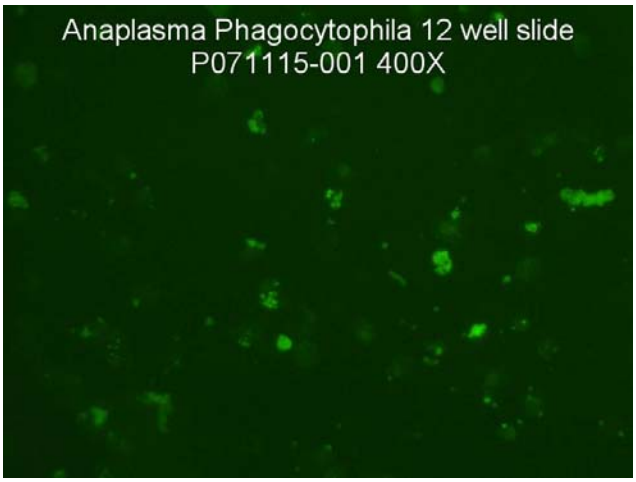
Certificate of Analysis

ANAPLASMA PHAGOCYTOPHILA

FA Substrate Slide



CATALOG NO.: SLD-IFA-AP
SIZE: 12 well
LOT: P071115-001
EXPIRATION DATE: 03 December 2009

AGENT: *Anaplasma phagocytophila*
Cell Culture Substrate: HL60 cells
Strain: NCH-1

QUALITY CONTROL METHOD: Indirect FA using *Anaplasma phagocytophila* positive control (catalog number 211-P-EE), *Anaplasma phagocytophila* negative control (211-N-EE), Anti-Equine IgG FITC Conjugate (catalog no. 043-10).

Specific Reaction: 4+ on positive cells. 10-30 infected cells per high power field with occasional free organism

Other Reactions or Comments: Trace background.

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 *A. phagocytophila* 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 *A. phagocytophila* in HL60 cells. Slides are unstained and sealed in moisture-free foil pouches.

INTERPRETATION OF RESULTS: Titers of 1:50 (IgG) and greater are considered positive. Sera positive at the 1:50 screening dilution should be rerun to determine their endpoint titer for comparison with earlier or later specimens from the same animal.

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 (1:50 for screening) 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 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.

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.