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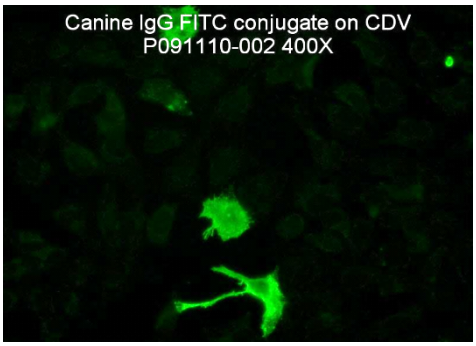
Certificate of Analysis

CANINE IgG

FITC Anti-Immunoglobulin Conjugate



CATALOG NO.: CJ-F-CANG-10ML
VOLUME: 10 ML
LOT: P091110-002
EXPIRATION: 15 September 2015

DESCRIPTION: Anti-Canine IgG polyclonal antiserum conjugated to fluorescein isothiocyanate (FITC). Caprine origin. Ready to use. Liquid.

QUALITY CONTROL METHOD: IFA using VMRD, Inc. Canine Distemper Virus (CDV) FA Substrate Slides (catalog no. SLD-IFA-CDV), and CDV IgG Positive Control (catalog no. PC-IFA-CDV-G).

Specific Reaction: 2-4+ with CDV IgG positive control and negative with SSDB and no background.

Other Comments: The raw material (concentrated conjugate) has also been screened by IFA and was found to react with *Toxoplasma gondii* @ 2-3+ and 1+ @ 1:4800 but does not react with *Anaplasma phagocytophila*, canine adenovirus type 2 (CAV-2), canine coronavirus (CCV), *Brucella canis* (canine brucellosis), canine distemper virus (CDV), canine herpesvirus type 1 (CHV-1), canine parainfluenza virus type 2 (CPI-2), canine parvovirus (CPV), *Ehrlichia canis*, *Borrelia burgdorferi* (Lyme disease), *Leishmania infantum*, *Neospora caninum* (canine origin), and *Rickettsia rikettsii* (RMSF).
Note: Goats commonly have antibodies to *Toxoplasma*. This goat-origin conjugate will bind directly to VMRD's *Toxoplasma gondii* substrate, catalog no. SLD-IFA-TOXO, making a negative serum appear positive; use catalog no. CJ-F-CANG-AP-1ML or CJ-F-CANG-AP-10ML (rabbit-origin affinity purified conjugate) instead.

PATTERN OF FLUORESCENCE: The pattern of fluorescence will vary depending on which canine system was used.

INTENDED USE: Systems listed above.

STORAGE: This conjugate is provided in liquid form and should be stored at 2-7°C. DO NOT FREEZE! It should also be stored in the original container and/or in the dark. If conjugate becomes cloudy it should be discarded. This conjugate contains 0.09% sodium azide as a preservative.

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.