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## **Certificate of Analysis**

### Neospora caninum (Canine)

IFA Positive Control Serum

CATALOG NO.: PC-IFA-NC-CAN VOLUME: 1 ml LOT: P100730-002 EXPIRATION: 26 April 2016

**AGENT:** Neospora caninum

# NC Canine Positive Control P100730-002 400X

**DESCRIPTION:** Canine serum diluted in PBS, 10% DBS, 0.09% sodium azide.

**QUALITY CONTROL METHOD:** IFA using VMRD, Inc. *Neospora caninum* 12-well slide (catalog no. SLD-IFA-NC), NC Canine Negative Control (catalog no. NC-IFA-NC-CAN), and Anti-Canine FITC conjugate (catalog no. CJ-F-CANG-1ML or 10ML).

**Specific Reaction:** 4+ fluorescence at neat with no background and an endpoint titer of 1/16.

**Other Comments: NA** 

**PATTERN OF FLUORESCENCE:** Organisms with bright diffuse or peripheral fluorescence.

**INTENDED USE:** As a positive control serum in detection of antibody to *Neospora caninum* by indirect FA technique. This serum should be used undiluted to demonstrate positive fluorescence.

**STORAGE:** Store at 2-7°C. DO NOT FREEZE! If control becomes cloudy it should be discarded.

**REFERENCES: NA** 

#### FOR IN VITRO LABORATORY USE ONLY.

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#### **RECOMMENDED STAINING PROCEDURE FOR INDIRECT FA:**

- 1. Warm slide to room temperature before removing from foil pouch.
- Place 50 μl diluted serum on the designated wells. Dilute serum in serum diluting buffer, pH 7.2 (catalog no. FASDB-100ML) however if high background due to anti-bovine IgG activity is present it may be advisable to use SSDB-100ML.
- 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. FARB-4X) 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 <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. FAMF-10ML) 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. FARB-4X) and then soak for 10 minutes in FA rinse buffer, pH 9.0.
- 5. 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.
- 6. Mount with mounting fluid [glycerol/FA rinse buffer, pH 9.0, (50/50)] (catalog no. FAMF-10ML) and view with good quality fluorescence microscope at 100X-250X. Confirmation may be at 400X.

#### SERUM DILUTING BUFFER (pH 7.2):\*

-	Na <sub>2</sub> HPO <sub>4</sub>
-	NaH <sub>2</sub> PO <sub>4</sub> 0.22 gm
	NaCl
-	BSA
-	DI/dH <sub>2</sub> OQ.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<sub>3</sub> if diluted serum is not going to be used within one week.

#### 4X FA RINSE BUFFER (pH 9.0):

- Na <sub>2</sub> CO <sub>3</sub>	11.4 gm
- NaHCO <sub>3</sub>	
- NaCl	8.5 gm
- DI/dH <sub>2</sub> O	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.