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Certificate of Analysis

TOXOPLASMA GONDII

IFA Negative Control Serum

CATALOG NO.: NC-IFA-TOXO-FEL VOLUME: 1 ml LOT: P111025-006 EXPIRATION: 17 November 2015

AGENT: Toxoplasma gondii

DESCRIPTION: Feline serum diluted in PBS, 1% BSA, 0.09% sodium azide.

QUALITY CONTROL METHOD: IFA using VMRD, Inc. *Toxoplasma gondii* 12-well slide (catalog no. SLD-IFA-TOXO), IgG Positive Control (catalog no. PC-IFA-TOXO-FEL-G), and Anti-Feline IgG FITC conjugate (catalog no. CJ-F-FELG-10ML).

Specific Reaction: Negative, no background with trace apical staining. **Other Comments:** NA

PATTERN OF FLUORESCENCE: None with our system.

INTENDED USE: As a negative control serum in detection of antibody to *Toxoplasma gondii* by indirect FA technique. This control serum should be used undiluted to demonstrate negative fluorescence, if any.

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.
- 2. Place 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 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 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 ₂ HPO ₄
-	NaH ₂ PO ₄ 0.22 gm
	NaCl
-	BSA
-	DI/dH ₂ 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% NaN₃ if diluted serum is not going to be used within one week.

4X FA RINSE BUFFER (pH 9.0):

- Na ₂ CO ₃	11.4 gm
- NaHCO ₃	
- NaCl	8.5 gm
- DI/dH ₂ 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.