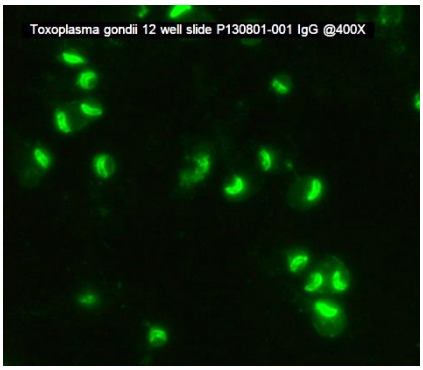
Veterinary Medical
Research & Development

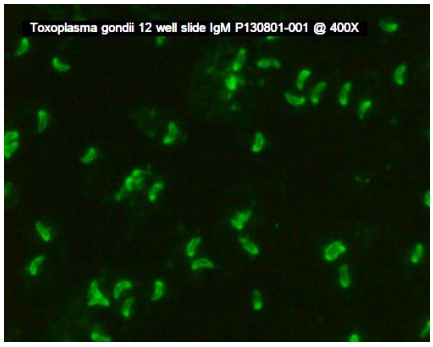
CERTIFICATE OF ANALYSIS

Toxoplasma gondii

FA Substrate Slide

Catalog No.:	SLD-IFA-TOXO
Size:	12 Well
Well Capacity:	10 µl
Lot:	P130801-001
Expiration:	25 September 2017
Agent:	*Toxoplasma gondii*
Strain:	RH
Cell Culture Substrate:	Vero-81 cells





Description:

Slides contain fixed Toxoplasma gondii from infected Vero cells spotted on the surface of Teflon-masked slides. The majority of the protozoa are extracellular. They are supplied fixed and unstained in moisture-free foil pouches.

Quality Control Method:

IFA using *Toxoplasma gondii* IgG positive control (catalog no. PC-IFA-TOXO-FEL-G), IgM positive control (catalog no. PC-IFA-TOXO-FEL-M), negative control (catalog no. NC-IFA-TOXO-FEL), anti-feline IgG FITC affinity purified conjugate (catalog no. CJ-F-FELG-AP-1ML or 10ML) and anti-feline IgM conjugate (catalog no CJ-F-FELM-AP-1ML or 10ML).

Specific Reaction: 4+ fluorescence with the IgG positive control, no background and 3+ with the IgM positive control, no background. Negative with the diluent control, no background. There were 50 to too numerous to count organisms per high-powered field.

Other Comments: When using Toxoplasma gondii systems it is recommended to use FASDB as a diluent.

Pattern Of Fluorescence:

Organisms with bright diffuse or peripheral fluorescence.

Interpretation Of Results:

Screening dilutions vary with species and must be determined by the investigator. Diffuse or peripheral staining is considered positive. As is the case with Neospora, apical (polar) staining is not considered positive.

Intended Use:

Generally used for Indirect FA to detect antibody to *Toxoplasma gondii* but may also be used as a positive and negative control substrate slide for Direct FA conjugates when applicable. May be used to differentiate antibody class (IgG or IgM) with suitable quality fluoresceinated second antibody conjugate.

Storage:

Store sealed in foil pouch at -20°C. Avoid self-defrosting freezers.

References: NA

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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 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. 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 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 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. FAMF-10ML) 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.

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