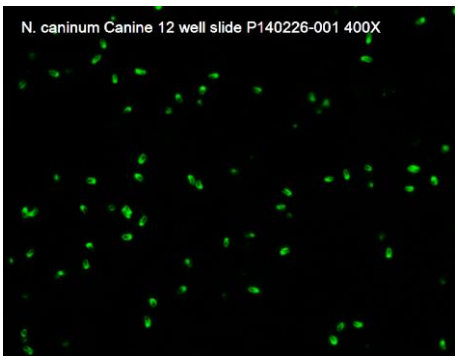
Veterinary Medical
Research & Development

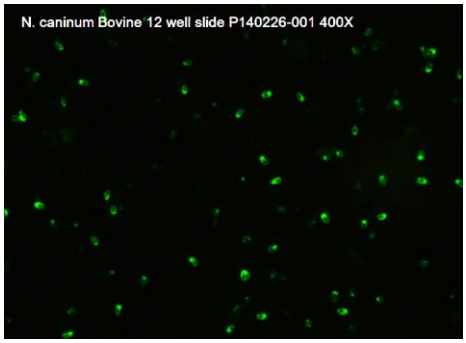
CERTIFICATE OF ANALYSIS

Neospora caninum

FA Substrate Slide

Catalog No.:	SLD-IFA-NC
Size:	12 Well
Well Capacity:	10 µl
Lot:	P140226-001
Expiration:	13 June 2018
Agent:	*Neospora caninum*
Strain:	NC-1
Cell Culture Substrate:	Vero cells





Description:

Wells contain Neospora caninum-infected Vero cultures spotted on the surface of Teflon-masked slides. The majority of the protozoa are extracellular. They are supplied fixed and unstained in moisture-free pouches.

Quality Control Method:

IFA using Neospora caninum bovine positive control (catalog no. PC-IFA-NC-BOV), bovine negative control (catalog no. NC-IFA-NC-BOV), and anti-bovine $IgG_{1,2}$ FITC affinity purified conjugate (catalog no. CJ-F-BOVG-AP-1ML or 10ML), canine positive control (catalog no. PC-IFA-NC-CAN), canine negative control (catalog no. NC-IFA-NC-CAN), and anti-canine IgG FITC conjugate (catalog no. CJ-F-CANG-1ML or 10ML).

Specific Reaction: 3-4+ fluorescence with the bovine positive control and 4+ with the canine positive control. Both the bovine and canine negative controls were negative with no background. There are 25 to 200 organisms per high-power field.

Other Comments: When using Neospora caninum systems it is recommended to use FA Serum Diluting Buffer with 1% BSA pH 7.2 (catalog no. FASDB-100ML) as a diluent.

Pattern Of Fluorescence:

Organisms with bright diffuse or peripheral fluorescence.

Interpretation Of Results:

Bovine sera should be screened at 1/200 for IgG. Canine sera should be screened at 1/50 for IgG. Diffuse or peripheral staining is considered positive. As is the case with *Toxoplasma*, apical (polar) staining is not considered positive.

Intended Use:

Generally used for Indirect FA to detect antibody to *Neospora caninum* but may also be used as a positive and negative control substrate slide for Direct FA conjugates when applicable.

Storage:

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

References:

Jenkins MC, Tuo W, Dubey JP. Evaluation of vaccination with Neospora caninum protein for prevention of fetal loss associated with experimentally induced neosporosis in sheep. Am J Vet Res. 2004 Oct;65(10):1404-8.

Dubey JP, Lindsay DS, Adams DS, et al. Serologic responses of cattle and other animals infected with Neospora caninum. Am J Vet Res. 1996 Mar;57(3):329-36.

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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.