

**VMRD**

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Certificate of Analysis

Anti-Neospora caninum Antiserum

CATALOG NO.: 210-70-NC
re-order as PAB-NC

VOLUME: 1 ml

LOT: P081121-006

EXPIRATION: 09 July 2016

AGENT: *Neospora caninum*

DESCRIPTION: Anti-*Neospora caninum* polyclonal antiserum. Goat origin.



QUALITY CONTROL METHOD: Indirect FA using VMRD, Inc. *Neospora caninum* 12-well FA Substrate slides (catalog no. SLD-IFA-NC); Anti-Goat IgG FITC Conjugate (catalog no. CJ-F-CAPG-10ML).

Specific Reaction: 3-4+ at 1/400 with no background. IFA endpoint = 1/6400.

Other Reactions or Comments: The antiserum P081121-006 has also been tested negative by VMRD's IFA systems for *Brucella canis*, canine adenovirus, canine coronavirus, canine distemper virus, canine herpesvirus, canine parainfluenza virus, canine parvovirus type 2, *Leishmania*, *Borrelia burgdorferi*, Rocky Mountain Spotted Fever, vesicular stomatitis virus, Bluetongue virus, bovine adenovirus types 1,3, and 5, infectious bovine rhinotracheitis, bovine leukemia virus, bovine parainfluenzavirus type 3, bovine parvovirus, bovine viral diarrhea virus, *Anaplasma phagocytophila*. A positive reaction is seen to *Ehrlichia canis* at an endpoint of 1/500, to *Toxoplasma gondii* at 1/800, to bovine respiratory syncytial virus at 1/50 and to reovirus at 1/50.

INTENDED USE: Useful for immunohistochemistry (procedure follows) and IFA.

STORAGE: Store at 2-7°C. This antiserum may also be frozen, but repeated freezing and thawing should be avoided. If conjugate becomes cloudy, it should be discarded. This antiserum contains 0.09% sodium azide as a preservative.

REFERENCES:

McAllister, M.M., *et al.* Oral inoculation of cats with tissue cysts of *Neospora caninum*. *AJVR* 59:441-444, 1998.

Helman, R.G., *et al.* Neosporal abortion in Oklahoma cattle with emphasis on the distribution of brain lesions in aborted fetuses. *J. Vet. Diagn. Invest.* 10:292-295, 1998.

Ramos-Vara, J.A., and M.E. Beissenherz. Optimization of immunohistochemical methods using two different antigen retrieval methods on formalin-fixed, paraffin-embedded tissues: Experience with 63 markers. *J. Vet. Diagn. Invest.* 12:307-311, 2000.

FOR IN VITRO LABORATORY USE ONLY.

WARRANTY: VMRD, Inc. warrants that this product is as described in the quantity and contents stated on the label at the time of delivery to the customer. NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE MADE BEYOND THE LABEL DESCRIPTION, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR USE. Remedy is limited to replacement of the product or refund of the purchase price. VMRD, Inc. is not liable for property damage, personal injury, or economic loss caused by the product. The information listed in this information sheet is provided for reference only, and should not be substituted for the user's own incoming material quality control.

IMMUNOHISTOCHEMISTRY: ABC PROCEDURE

Microprobe System (Fisher Scientific, catalog no. 15-188-20)

Tissues to be stained should be formalin fixed and embedded in paraffin.

1. Deparaffinize in xylene or Pro-par 10 minutes
2. Absolute alcohol 3X rinses
3. 3% hydrogen peroxide in methanol 5 minutes, RT
(135 ml methanol + 15 ml 30% H₂O₂)
4. 95%, 80%, 70% EtOH to DW Rinse well
5. 0.1% Pronase 10 minutes, 37°C
[0.05 gm Protease (Sigma P-5147) + 50 ml Tris buffer]
6. Wash in running deionized water 5 minutes
7. TTBS-HS/Tx Rinse well
8. Place slides in microprobe holder
9. Block with 5% normal rabbit serum made up in 0.1% 20 minutes, 37°C
gelatin (diluent is TTBS-HS/Tx)
10. **BLOT** only
11. Primary antibody diluted in blocker 30 minutes, 37°C
VMRD catalog no. 210-70-NC; use at 1:2000
12. TTBS-HS/Tx Rinse well
13. Secondary antibody diluted in TTBS-Hs/TX 5:1000 20 minutes, 37°C
Vectastain peroxidase goat IgG kit (Vector Labs, Pk-4005)
14. TTBS-HS/Tx Rinse well
15. ABC reagent 20 minutes, 37°C
Dilute in TTBS-Hs/TX
10(A) + 10(B):1000
Vectastain, peroxidase goat IgG kit (Vector Labs, Pk-4005)
16. TTBS-HS/Tx Rinse well
17. Chromogen substrate Use according to
BioGenex AEC 0.001 ml + 0.1 ml H₂O₂ in acetate buffer manufacturer's directions, RT
18. Rinse well in running deionized water 5 minutes
19. Counterstain in Mayer's Hematoxylin 2 minutes
20. Tap water rinse 5 minutes
21. Mount with GelTol (aqueous mounting media), Shandon Lipshaw Co. (Lerner Labs), catalog no. 484950

RESULTS:

Specific - Red

Background - Blue

Always compare with negative control for non-specific staining.

May have weak cross-reactivity with other protozoa.

SOLUTIONS:

Tris Buffer 0.05 M, pH 7.6

| | |
|-----------------------------------|---------|
| Trizma-HCl (Sigma T-3253) | 3.03 gm |
| Trizma base (Sigma T-1503) | 0.69 gm |
| Dissolve above in deionized water | 500 ml |

TTBS-HS/Tx

| | |
|----------------------------|----------|
| Trizma-HCl (Sigma T-3253) | 15.15 gm |
| Trizma base (Sigma T-1503) | 3.47 gm |
| Deionized water | 1000 ml |
| NaCl | 20.29 gm |
| 10% Triton-X (Sigma X-100) | 2.5 ml |

SUPPLIERS:

BioGenex Laboratories
4600 Norris Canyon Rd.
San Ramon, CA 94583
(510) 275-0550
Fax (510) 725-1999

Shandon Lipshaw Co. (Lerner Labs)
171 Industry Dr.
Pittsburgh, PA 15275
(800) 245-6212
Fax (412) 788-1138

Vector Labs
30 Ingold Rd.
Burlingame, CA 94010
(415) 697-3600
Fax (415) 697-0339

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