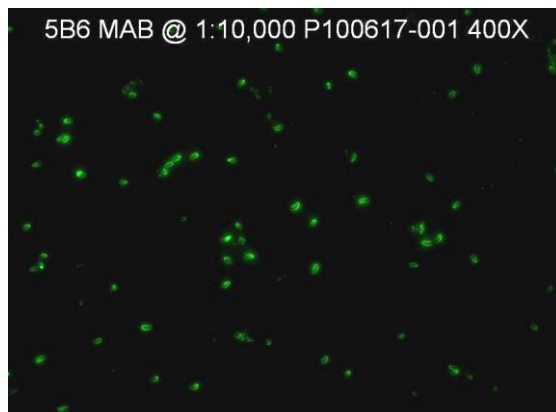


CERTIFICATE OF ANALYSIS

5B6-25

Monoclonal Antibody

<b>Catalog No. / Cell Line:</b>	5B6-25
<b>Lot:</b>	P100617-001
<b>Isotype:</b>	IgG <sub>1</sub>



**Specificity:**

Recognizes as a binding epitope a 65 kDa glycosylated protein of *Neospora caninum*.

**Known Applications:**

ELISA, postmortem immunohistochemical analysis, indirect immunofluorescence, Western blot and dot blot.

**Description:**

This monoclonal antibody is produced as mouse ascites fluid, clarified by centrifugation, and filtered through a 0.2 µm filter. The antibody concentration is 1.0 mg/ml, in phosphate-buffered saline, stabilized with 4 mg/ml bovine serum albumin (BSA) and preserved with 0.09% sodium azide.

**Quality Control Method:**

IFA using VMRD, Inc. *N. caninum* 12 well slide (catalog no. SLD-IFA-NC), Isotype IgG<sub>1</sub> Control, and Anti-Mouse IgG FITC Conjugate (catalog no. CJ-F-MURG-AP-1ML or 10ML).

**Specific Reaction:** 4+ fluorescence at 0.1 µg/ml with an endpoint concentration less than 0.01 µg/ml. The isotype control was negative with 2+ apical.

**Other Comments:** NA

**Pattern of Fluorescence:**

Organisms with bright diffuse or peripheral fluorescence.

**Storage:**

When the vial is stored at 2-7°C, it should be stable for one year.

**References:**

Baszler TV, Adams DS, Vander-Schalie J, et al. Validation of a Commercially Available Monoclonal Antibody-Based Competitive-Inhibition Enzyme-Linked Immunosorbent Assay for Detection of Serum Antibodies to *Neospora caninum* in Cattle. *J Clin Microbiol* 2001;39(11):3851-3857.

**Immunohistochemistry Procedure for  
*Neospora caninum* (gp65) Monoclonal Antibody 5B6**  
[courtesy of Washington Animal Disease Diagnostic Laboratory]

<b>AGENT</b>	<i>Neospora caninum</i>
<b>CLINICAL DISEASE</b>	Bovine abortion
<b>HYDROGEN PEROXIDE BLOCK</b>	3% in water <b>after antigen retrieval step</b> / 5 minutes / room temperature
<b>ANTIGEN RETRIEVAL BUFFER</b>	0.06% Pronase (Sigma P-5147) / 10 minutes / warm
<b>ANTIBODY DILUENT</b>	Dako tris-buffered saline (TBS) 1% filtered bovine serum albumin (BSA)
<b>PRIMARY – POSITIVE</b>	5B6 monoclonal antibody (use at 1.25 µg/ml—dilute in 1% BSA) / 45 minutes / room temperature
<b>PRIMARY – NEGATIVE</b>	Isotype-matched (IgG <sub>1</sub> ) monoclonal antibody (use at 1.25 µg/ml—dilute in 1% BSA) / 45 minutes / room temperature
<b>LABELED POLYMER</b>	Dako Envision + K4001 anti-mouse HRP-labeled polymer, / 30 minutes / room temperature
<b>CHROMOGEN</b>	Dako K3464 ready-to-use AEC chromogen / 1X 5 minutes / room temperature
<b>POSITIVE CONTROL TISSUE</b>	Cell culture zoites / formalin-fixed / paraffin-embedded

**NOTES:** Percentage of positive foci is low in tissues; replicate sections may be required.  
DOES NOT cross-react with *Toxoplasma* or *Sarcocystis*.