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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Monoclonal Anti-*Mycobacterium tuberculosis* GlnA (Gene Rv2220), Clone B (9D9-G12) (produced *in vitro*)

# Catalog No. NR-50108

This reagent is the tangible property of the U.S. Government.

# **Product Description:**

Antibody Class: IgG<sub>3</sub>λ

Monoclonal antibody to *Mycobacterium tuberculosis*, strain H37Rv glutamine synthetase (GlnA; Rv2220), clone B (9D9-G12) was produced in cell culture using a B cell hybridoma generated by the fusion of myeloma cells with immunized mouse splenocytes.

# Lot: 70006606

# Manufacturing Date: 30JAN2019

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 19.anti-GInA.B.1.8.45.mm is attached.

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#### Work Sheet for Antibodies

# **General Information**:

BEI Catalog Number: NR-50108 Product Description: Anti-GlnA (Rv2220, Clone B) CSU Lot Number: 19.anti-GlnA.B.1.8.45.mm Species: *Mycobacterium tuberculosis* Strain: H37Rv Type (select one): <u>X</u> Mouse Monoclonal \_\_\_\_\_\_Rabbit Polyclonal

Guinea Pig Polyclonal

#### **Production Information:**

Cell Line:<u>19.anti-GlnA.B.1.15.45.Hyb</u>SOP#:<u>AB103.5, AB104.4</u>Notebook/pp: <u>Monoclonal Antibody #4</u><u>BEI, pgs. 1, 3-5,13-16, 20-21, 23-24, 26-58</u>Amount of CS Harvested: ~41.5mLClarity: clear (after centrifugation and filtration)Additional Notes:<u>Cell debris was removed by centrifugation and filtration.</u>IgG Purification:N/AIg isotype:<u>IgG3 (heavy chain), Lambda (light chain)</u>SOP#:<u>AB106</u>Notebook/pp:<u>Monoclonal Antibodies #4 BEI, pg. 59</u>

# **QC Information:**

 Tested Against: <u>1μg ovalbumin conjugated-peptide, 1μg recombinant GInA (17.rEC.02.07.17.RGInA), 5μg Whole</u>

 <u>Cell Lysate (15.Rv.9.17.15.WCL)</u>

 SOP#:
 <u>AB102.1, SP039.1</u>

 Notebook/pp:
 <u>Monoclonal Antibodies #4 BEI, pg. 57-58</u>

 Tested by: Western blot:
 X

 ELISA:
 X

 Titer:
 1: 1000

 Special Instructions:
 3% BSA recommended for blocking.

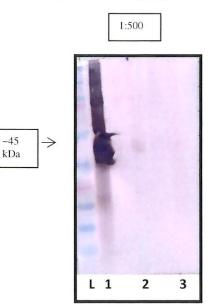
# QC ELISA:

Date performed: 01/09/19

Titer	30 minutes, 1µg conjugated ovalbumin conjugated-peptide (average)	30 minutes, 1µg recombinant GInA (average)	30 minutes, 5µg WCL (average)
1: 1000	1.430	0.08	0.094
Positive control (Ova- conjugated peptide) = M#2 1:5000 Second (final) bleed 11/12/18	0.8255	N/A	N/A
Positive control (recombinant GlnA) = M#6 1:1000 Final bleed	N/A	3.328	N/A
Positive control (WCL) = 1:40,000 12.Anti- WCL.8.20.rp 9/20/12	N/A	N/A	OVERFLOW
Negative Control = TBST	0.0845	0.0775	0.088

Note: Antibody reactive against linear peptide. Therefore, if running an ELISA for native protein, a denatured ELISA protocol should be used<sup>1</sup>.

# QC Western blot: Date performed: 01/09/18

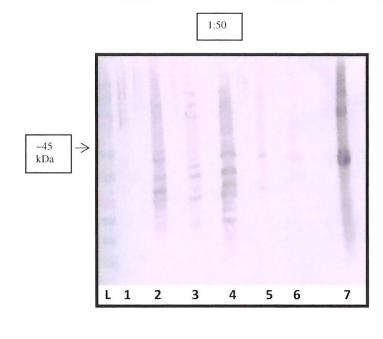


- L. Ladder
- 1. 1µg Ovalbumin conjugated-peptide
- 2. 1µg Recombinant GlnA
- 3. 5µg Whole Cell Lysate

#### Western blot against subcellular fractions:

Tested Against: <u>1μg ovalbumin conjugated-peptide</u>, <u>1μg recombinant GlnA (17.rEC.02.07.17.RGlnA)</u>, <u>5μg CFP (11.Rv.2.2.21.5.CFP, NR-14825)</u>, <u>5μg Whole Cell Lysate (15.Rv.9.17.15.WCL, NR-14822)</u>, <u>5μg Cytosol (08.Rv.2.5.21.7.CYT)</u>, <u>5μg Membrane (08.Rv.2.5.21.7.MEM)</u>, <u>5μg Cell Wall (14.Rv.2.12.9.CW, NR-14828)</u> Notebook/pp: <u>Monoclonal Antibodies #4 BEI, pgs. 59, 63</u> Date performed: 01/25/19

Notes: <u>A titer of 1:50 was used to compare all of the subcellular fractions.</u>



L	Ladder
1	5μg CFP
2	5µg Whole Cell Lysate
3	5µg Cytosol
4	5µg Membrane
5	5µg Cell Wall
6	1µg recombinant GlnA
7	1µg ovalbumin conjugated-peptide

Aliquot Information: 40 vials x 0.5mL (BEI), 36 vials x 0.5mL (In-house)

30/19 (Research Associate) (date)

(Laboratory Supervisor (date)

# References:

 Hnasko, R., Lin, A., McGarvey, J.A., & Stanker, L.H. (2011). A rapid method to improve protein detection by indirect ELISA. *Biochemical and Biophysical Research Communications*, 410, Issue 4, 726-731.