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Anti-Mycobacteria mAb (LAM antibody)

CODE No. D372-3

CLONALITY Monoclonal
CLONE TMDU3
ISOTYPE Mouse IgM κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgM from mouse ascites fluid
IMMUNOGEN Fractionated mycobacteria by anion-exchange chromatography
FORMULATION PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Western blotting 5 µg/mL
Immunohistochemistry 1 µg/mL (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 40 min. at 97°C in 10 mM citrate buffer (pH 6.2)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Other
Sample	Not tested	Not tested	Not tested	Lipoarabinomannan (LAM) from *Mycobacterium tuberculosis* Aoyama-B
Reactivity				+

REFERENCES

1) Yang, G., *et al.*, *Neuropathology* **38**, 159-164 (2018)
2) Sakakibara, Y., *et al.*, *Respiration* **93**, 264-270 (2017)
3) Iida, T., *et al.*, *J. Mycobac. Dis.* **4**, 142 (2014)

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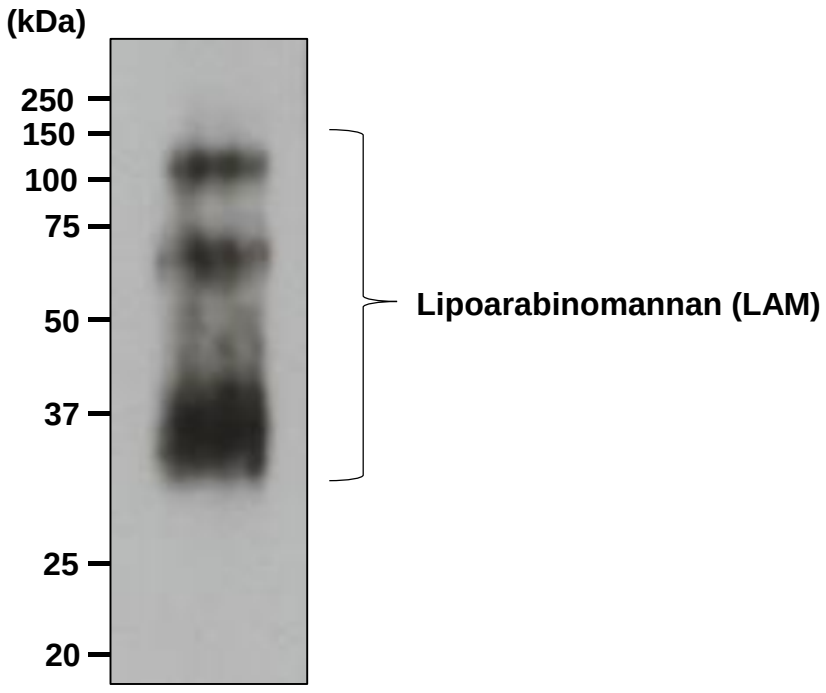
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Mix the sample with equal volume of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the sample for 3 min. and centrifuge. Load 20 μL (10 μg) of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Lipoarabinomannan from *Mycobacterium tuberculosis* Aoyama-B)



Western blot analysis of Lipoarabinomannan from Mycobacterium tuberculosis Aoyama-B

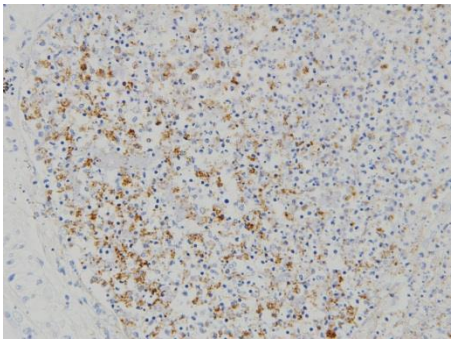
Immunoblotted with Anti-Mycobacteria mAb (LAM antibody) (D372-3)

Immunohistochemistry for formalin fixed paraffin-embedded section

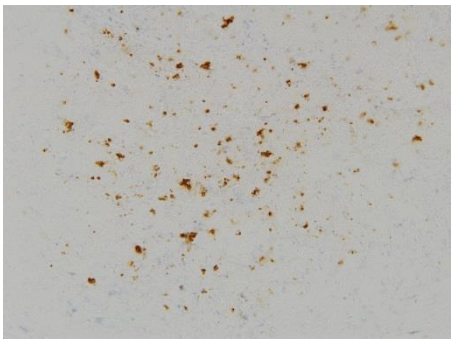
1) Deparaffinize tissue sections in Xylene 3 times for 3 min. each.
2) Immerse the slides with Ethanol 3 times for 3 min. each, then wash the slides in PBS 3 times for 3 min. each.
3) Remove the slides from PBS and heat-treat with 10 mM Citrate buffer (pH 6.2) for 40 min. at 97°C using microwave oven.
4) Let the slide cool down until at room temperature in the Citrate buffer.
5) Remove the slides from the Citrate buffer and inactivate endogenous peroxidase with 3% H_2O_2 in Methanol for 10 min.
6) Wash the slides with PBST [0.25% Tween-20 in PBS] 3 times for 5 min. each.
7) Incubate the sections with 2.5% normal horse serum (Vectastain Universal Elite ABC Kit, Vector Laboratories; code no. PK-7200) for 30 min. at room temperature to block non-specific staining. Do not wash.
8) Incubate the sections with primary antibody diluted with DAKO REAL Antibody diluent (Dako; code no. S2022) as suggested in the **APPLICATIONS** overnight at room temperature. (The concentration of antibody will depend on the conditions.)
9) Wash the slides 3 times in PBST for 5 min. each.
10) Incubate the sections with Biotinylated anti-mouse/rabbit IgG (Vectastain Universal Elite ABC Kit) for 30 min. at room temperature.
11) Wash the slides 3 times in PBST for 5 min. each.
12) Incubate the sections with ABC reagent (Vectastain Universal Elite ABC Kit). Incubate for 30 min. at room temperature.
13) Wash the slides 3 times in PBST for 5 min. each.
14) Visualize by reacting for 8 min. with Histofine Simplestain DAB Solution (Nichirei Biosciences; code no. 415171). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Wash the slides 2 times in PBS for 5 min. each.
16) Counterstain in hematoxylin for 5 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
17) Dehydrate by immersing in Ethanol 3 times for 5 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Pulmonary tuberculosis lung tissue)

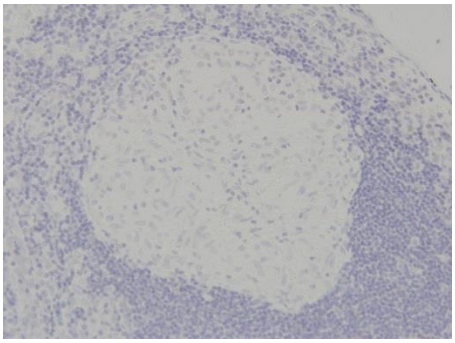
Pulmonary Tuberculosis



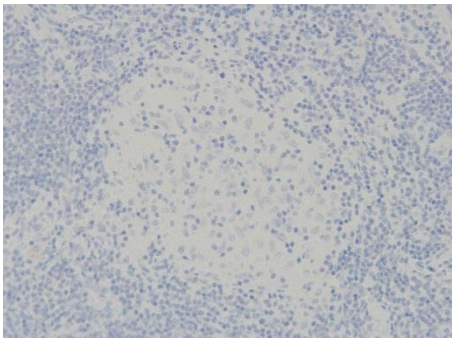
Pulmonary Tuberculosis (caseous necrosis)



Sarcoid granuloma



Sarcoid reaction granuloma



Immunohistochemical detection of Lipoarabinomannan (LAM) in pulmonary tuberculosis lung tissue.

Brown: Anti-Mycobacteria mAb (LAM antibody) (D372-3)
Blue: Hematoxylin

The data were kindly provided by Prof. Yoshinobu Eishi[1] and Mr. Keisuke Uchida[2].
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