

Human AIM / CD5L / Sp α Rabbit Polyclonal Antibody

Cat# CY-P1036

100 μ g (0.8 mg/mL x 125 μ L)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB	H	38-40 kDa	Rabbit IgG

Background

AIM, also called CD5L/Sp α , is a soluble 38 or 40-kDa glycoprotein expressed by macrophages present in lymphoid tissues (spleen, lymph node, thymus, and bone marrow), for which little functional and structural information is available. It belongs to the group B of the scavenger receptor cysteine-rich (SRCR) superfamily that includes the lymphocyte surface receptors CD5 and CD6 among others. AIM is able to bind to not only myelomonocytic and lymphoid cells, but also the surface of several Gram-positive and Gram-negative bacterial strains, which suggests that it may play an important role in the regulation of the innate and adaptive immune systems. It has been found present in IgM but not IgG or IgA fractions from human serum. AIM is a circulating protein that may play a role in the homeostasis of IgM antibodies. In addition, it has been reported that AIM expression is induced in mouse macrophages in response to loading with highly oxidized low density lipoprotein (oxLDL), and that AIM is expressed in foam cells within atherosclerotic lesions.

Specificity/Sensitivity: This antibody detects endogenous levels of AIM/CD5L/ Sp α .

Source/Purification: This antibody is produced by immunizing rabbit with a recombinant human full-length AIM/CD5L/Sp α which was produced by *E. coli*. IgG is purified by immunoaffinity chromatography using mammalian-expressed recombinant AIM/CD5L/Sp α .

Recommended Antibody Dilutions: Western blotting: 0.5-1 μ g/mL

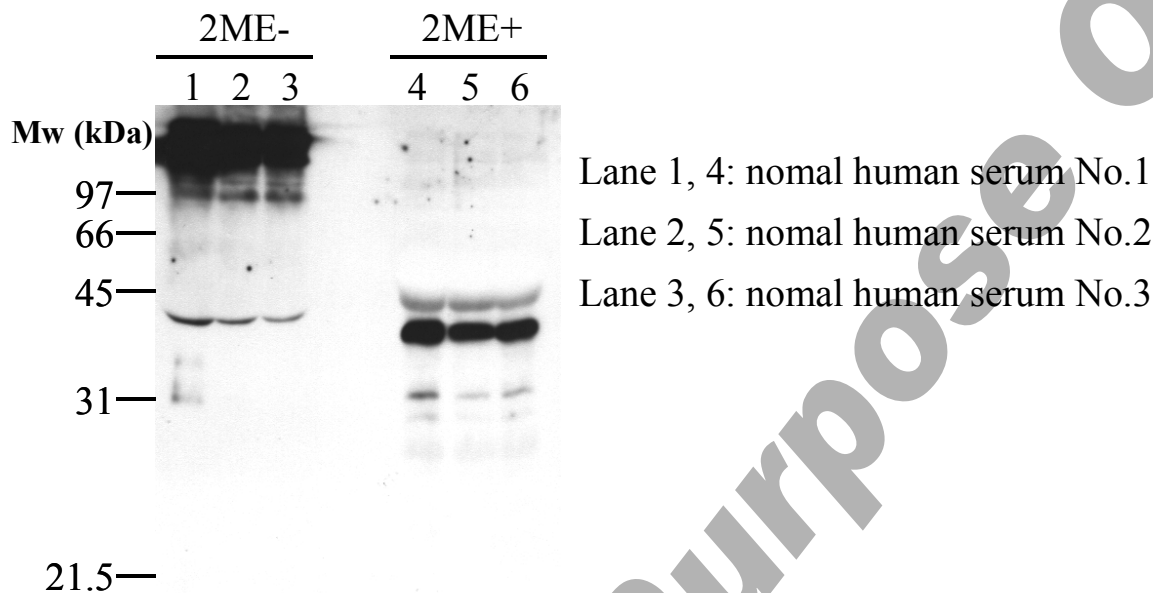
Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western IP: Immunoprecipitation IHC: Immunohistochemistry IC: Immunocytochemistry F: Flow cytometry E: ELISA FP: Fluorescence Polarization assay

Species Cross-Reactivity Key: H: human M: mouse R: rat Hm: hamster Mk: monkey Mi: mink C: chicken X: *Xenopus* Z: zebra fish All: all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

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Fig.1 Western blot analysis of Human AIM/CD5L/Sp α 

Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)

SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red

Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).

10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).

Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 mL water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 μ L Tween-20 (100%).

Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 μ L per well of 6-well plate or 500 μ L per plate of 10 cm² plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 μ L sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 μ L onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

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