Anti-human NTAL Antibody

ORDERING INFORMATION

Catalog Number: AF4066

Lot Number: YJI01

Size: 100 µg

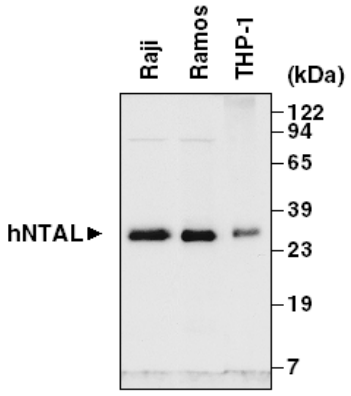
Storage: -20° C

Specificity: human NTAL

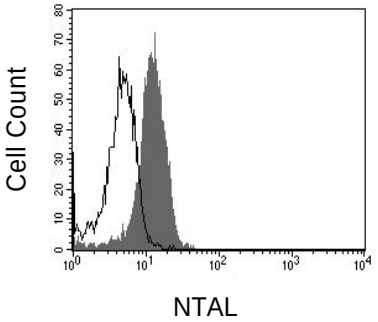
Immunogen: *E. coli*-derived recombinant human NTAL (rhNTAL; aa 1 - 243)

Ig Type: sheep IgG

Applications: Western blot
Flow cytometry



Sheep anti-NTAL figure legend:
Whole cell extracts from exponentially growing Ramos, Raji, and THP-1 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 1.0 µg/mL sheep anti-NTAL antibody.



THP-1 cells were stained with anti-NTAL (R&D Systems, Cat. # AF4066, filled histogram), or control antibody (R&D Systems, Cat. # 5-001-A, open histogram) followed by NL637™-conjugated anti-sheep IgG (R&D Systems, Cat. # NL011).

Background

Non-T cell activation linker (NTAL), also known as linker for activation of B cells (LAB), is a transmembrane adaptor protein involved in immunoreceptor signaling. NTAL is expressed in lipid raft microdomains of B cells, mast cells, monocytes and NK cells. Rapid tyrosine phosphorylation of NTAL occurs upon BCR aggregation in B cells, FcεRI aggregation and Kit activation in mast cells, and FcγRI aggregation in monocytes. Phosphorylated NTAL recruits signaling molecules such as Grb2, Gab1, and c-Cbl into receptor-signaling complexes. Defects in the NTAL gene may cause Williams-Beuren syndrome, a rare genetic disorder characterized by mild mental retardation, and abnormalities in the cardiovascular and musculo-skeletal systems.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived, recombinant human NTAL (rhNTAL; aa 1 - 243; GenBank Accession # AAH09204). Polyclonal antibody was affinity-purified on a column derivatized with rhNTAL and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) containing 5% trehalose.

Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for 12 months from date of receipt when stored at -20° C to -70° C. The reconstituted antibody should be aliquoted and stored at -20° C **in a manual defrost freezer** for 12 months without detectable loss of activity**. Avoid repeated freeze/thaw cycles.**

Specificity

The antibody detects human NTAL in the applications listed below.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution
25 mM Tris, pH 7.5	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer
0.05% Tween® 20	pH to 7.5

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Blocking Solution containing 1.0 µg/mL sheep anti-NTAL antibody.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:2,000 dilution of HRP-conjugated donkey anti-sheep Ig (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent detection reagents (R&D Systems, Catalog # AR004) or equivalent.

Cell lysates for Western blotting - To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and sonicate with a probe sonicator using 3 - 4 bursts of 5 - 10 seconds each. Heat extracts in a boiling water bath for 5 minutes and load onto polyacrylamide gels. Samples may be diluted with 1X SDS sample buffer to the desired concentration.

Flow cytometry - This antibody was tested in flow cytometry using THP-1 cells. For intracellular staining to detect NTAL, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 5 x 10^5 cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-sheep IgG conjugated to a fluorochrome.

Optimal dilutions should be determined by each laboratory for each application.

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