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Product datasheet for AM26636AF-N

Anpep Rat Monoclonal Antibody [Clone ID: 123H1]

Product data:

Product Type:	Primary Antibodies
Clone Name:	123H1
Applications:	FC, IP
Recommended Dilution:	**Immunoprecipitation:** 5 µg/1000 µl of cell extract from 5x10^6 cells. **Flow Cytometry:** 5-10 µg/ml (final concentration). For details see ***Protocol*** below.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Murine DC from C57BL/6 mice
Specificity:	This antibody recognizes Mouse CD13. Other species not tested.
Formulation:	PBS containing 50% Glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein G Agarose Chromatography
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	alanyl (membrane) aminopeptidase
Database Link:	Entrez Gene 16790 Mouse P97449

View online »

Background: CD13 is a myeloid differentiation molecule expressed on committed myeloid progenitors, granulocytes, monocytes, and leukemic cells of myeloid origin. It is also expressed on nonhematopoietic cells including fibroblasts, renal proximal tubule, and small intestine brush-border membrane. The gene for CD13 has recently been cloned the cDNA sequence shows that CD13 is the metalloprotease aminopeptidase N. Biochemical studies have shown that CD13 is a 150-kDa glycoprotein, which exists as a 130-kDa intracellular precursor form. This 130-kDa precursor molecule is post-translationally modified in the Golgi apparatus to produce the 150-kDa mature cell surface form of the molecule.

Synonyms: Aminopeptidase N, ANPEP, APN, PEPN

Note: This product was originally produced by MBL International.

Protocol:

Flow Cytometric analysis for floating cells

Protocol 1

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25°C).
5) Add 30 µL of anti-Mouse CD13 (123H1) (5 µg/mL) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature (20~25°C).
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
7) Add 30 µL of 1:40 FITC conjugated anti-rat IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature (20~25°C).
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.
Positive controls for flow cytometry; JAWSII, C2C12

Protocol 2

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with PBS containing 25% normal goat serum, 1 mg/mL normal human IgG and 0.1% NaN3 (5x10e6 cells/mL).
3) Add 20 µL of anti-Mouse CD13 monoclonal antibody (123H1) (50 µg/mL) diluted with the washing buffer into each tube.
4) Add 50 µL of the cell suspension into each tube. Mix well and incubate for 30 minutes at

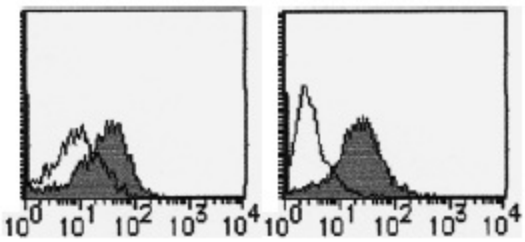
room temperature (20~25 oC).
5) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
6) Resuspend the cells with 50 µL of the washing buffer.
7) Add 30 µL of 1:40 FITC conjugated anti-rat IgG diluted with the washing buffer into each tube. Mix well and incubate for 15 minutes at room temperature (20~25°C).
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.
Positive controls for flow cytometry; JAWSIIv, C2C12

Immunoprecipitation

1) Wash the Biotin labeled JAWS? cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the APPLICATIONS into 1000 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein G agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the agarose 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
8) Incubate the membrane with 1:20,000 HRP conjugated Streptavidin diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions.)
9) Wash the membrane with PBS (5 minutes x 6 times).
10) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagents for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose to X-ray film in a dark room for 5 minutes. Develop the film as usual. The conditions for exposure and development may vary.
Positive control for immunoprecipitation; JAWSII

Product images:

Immunoprecipitation of Mouse CD13 from JAWSII cells with Rat IgG2b (1) or AM26636AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26636AF-N.



Flow cytometric analysis of Mouse CD13 expression on JAWSII cells (left) and C2C12 cells (right). Open histogram indicates the reaction of Isotypic control to the cells. Shaded histogram indicates the reaction of AM26636AF-N to the cells.