Anti-CD73 Antibody [A6F9]

HA601011

Product Type:	Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, FC, IF-Cell
Molecular Wt:	Predicted band size: 63 kDa
Clone number:	A6F9

Description: 5'-nucleotidase (5'-NT), also known as ecto-5'-nucleotidase or CD73 (cluster of differentiation 73), is an enzyme that in humans is encoded by the NT5E gene. CD73 commonly serves to convert AMP to adenosine. Ecto-5-prime-nucleotidase catalyzes the conversion at neutral pH of purine 5-prime mononucleotides to nucleosides, the preferred substrate being AMP. The enzyme consists of a dimer of 2 identical 70-kD subunits bound by a glycosyl phosphatidyl inositol linkage to the external face of the plasma membrane. The enzyme is used as a marker of lymphocyte differentiation. Consequently, a deficiency of NT5 occurs in a variety of immunodeficiency diseases. Other forms of 5-prime nucleotidase exist in the cytoplasm and lysosomes and can be distinguished from ecto-NT5 by their substrate affinities, requirement for divalent magnesium ion, activation by ATP, and inhibition by inorganic phosphate. Rare allelic variants are associated with a syndrome of adult-onset calcification of joints and arteries (CALJA) affecting the iliac, femoral, and tibial arteries reducing circulation in the legs and the joints of the hands and feet causing pain. NT5E can act as an immune inhibitory control molecule. Free adenosine generated by NT5E inhibits cellular immune responses and thereby promotes immune escape of tumor cells.

Immunogen:	Recombinant protein within Human CD73 27-549.
Positive control:	U-87 MG cell lysate, A375 cell lysate, 4T1 cell lysate, NCI-H441 xenograft tissue, U-87 MG xenograft tissue, human liver tissue, mouse liver tissue, rat liver tissue, U-87 MG.
Subcellular location:	Cell membrane.
Database links:	SwissProt: P21589 Human | Q61503 Mouse | P21588 Rat

Recommended Dilutions:

WB	1:2,000
IHC-P	1:200-1:1,000
IF-Tissue	1:100
FC	1:500-1:1,000
IF-Cell	1:100

Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein G affinity purified.

Images

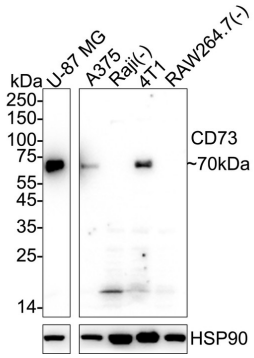

Fig1: Western blot analysis of CD73 on different lysates with Mouse anti-CD73 antibody (HA601011) at 1/2,000 dilution.

Lane 1: U-87 MG cell lysate
Lane 2: A375 cell lysate
Lane 3: Raji cell lysate (negative)
Lane 4: 4T1 cell lysate
Lane 5: RAW264.7 cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 63 kDa
Observed band size: 70 kDa

Exposure time: Lane 1: 40 seconds; Lane 2-5: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA601011) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Anti-Mouse IgG for IP Nano-secondary antibody (NBI02H) at 1/5,000 dilution was used for 1 hour at room temperature.

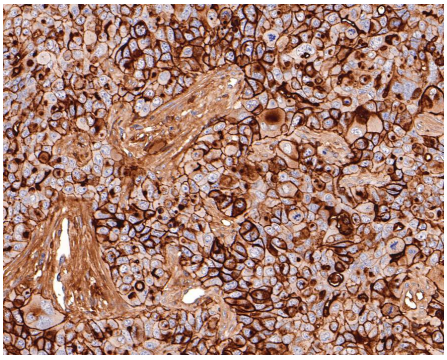

Fig2: Immunohistochemical analysis of paraffin-embedded NCI-H441 xenograft tissue with Mouse anti-CD73 antibody (HA601011) at 1/600 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601011) at 1/600 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

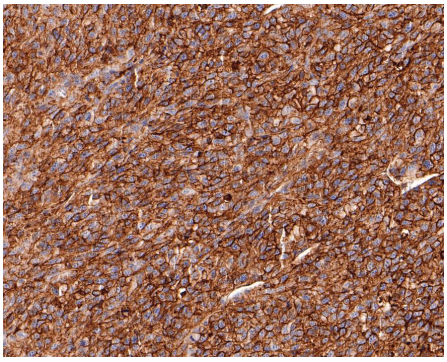

Fig3: Immunohistochemical analysis of paraffin-embedded U-87 MG xenograft tissue with Mouse anti-CD73 antibody (HA601011) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601011) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

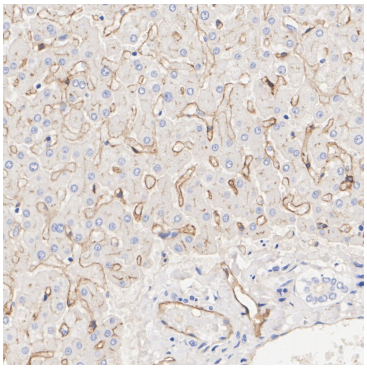

Fig4: Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-CD73 antibody (HA601011) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601011) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

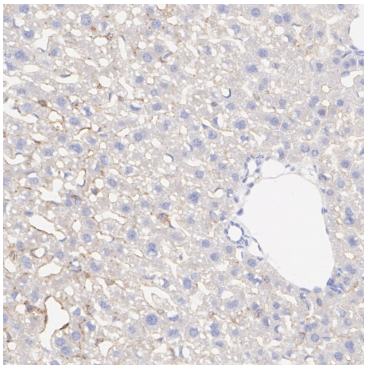

Fig5: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Mouse anti-CD73 antibody (HA601011) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601011) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

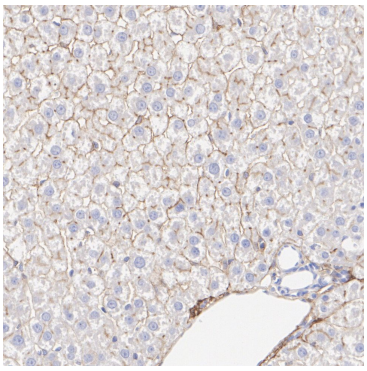

Fig6: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Mouse anti-CD73 antibody (HA601011) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA601011) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

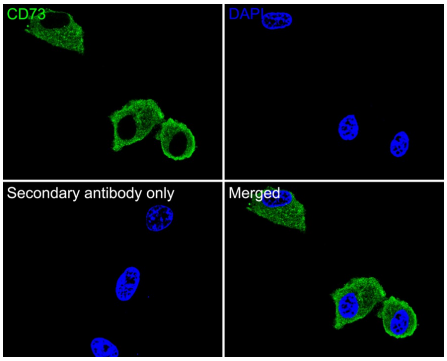

Fig7: Immunocytochemistry analysis of U-87 MG cells labeling CD73 with Mouse anti-CD73 antibody (HA601011) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Mouse anti-CD73 antibody (HA601011) at 1/100 dilution in 2% BSA overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

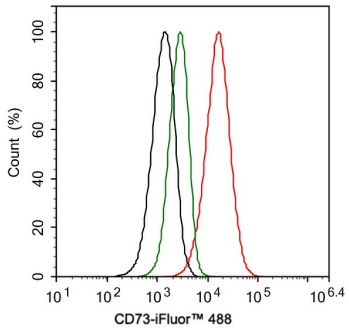

Fig8: Flow cytometric analysis of U-87 MG cells labeling CD73.

Cells were fixed and permeabilized.Then stained with the primary antibody (HA601011, 1ug/ml) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Harvey JB. et. al. CD73's Potential as an Immunotherapy Target in Gastrointestinal Cancers. Front Immunol. 2020 Apr
2. Neo SY. et. al. CD73 immune checkpoint defines regulatory NK cells within the tumor microenvironment. J Clin Invest. 2020 Mar