abcam

ab317462

Anti-CD73 antibody [RM2050]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Multiclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	RM2050
Purification technique	Affinity purification Protein A
Specificity	Unsuitable for mouse IHC.
Concentration	0.511 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/500 - 1/5000
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse
Dilution info	1/30
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information NT5E

Function	Catalyzes the hydrolysis of nucleotide monophosphates, releasing inorganic phosphate and the corresponding nucleoside, with AMP being the preferred substrate (PubMed:21933152, PubMed:22997138, PubMed:23142347, PubMed:24887587, PubMed:34403084). Shows a preference for ribonucleotide monophosphates over their equivalent deoxyribose forms (PubMed:34403084). Other substrates include IMP, UMP, GMP, CMP, dAMP, dCMP, dTMP, NAD and NMN (PubMed:21933152, PubMed:22997138, PubMed:23142347, PubMed:24887587, PubMed:34403084).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What are recombinant multiclonals?
Recombinant multiclonals are a mixture of recombinant antibodies co-expressed from a library of heavy and light chains. They offer several advantages including:

- The sensitivity of polyclonal antibodies by recognising multiple epitopes
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity

- Long-term security of supply
- Animal-free batch production

View our range of recombinant multiclonal antibodies.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	CD73 also known as ecto-5'-nucleotidase is an enzyme that functions at the cellular level. The CD73 protein hydrolyzes extracellular nucleotides into nucleosides by removing phosphate groups. It is a 70 kDa glycosylphosphatidylinositol-anchored molecule found on the surface of various cell types including endothelial cells lymphocytes and epithelial cells. Its presence is noted in a wide range of tissues across the human body highlighting its versatile role in cellular communication and metabolism.
Biological function summary	CD73 participates in the regulation of purinergic signaling through adenosine production. It acts in cooperation with other cell surface enzymes such as CD39 forming a functional complex that handles ATP breakdown to adenosine. This enzymatic activity modulates immune responses tissue protection and inflammation control. In addition to immune-related functions CD73 supports the maintenance of vascular integrity and proper cellular adhesion which is important in different physiological and pathological contexts.
Pathways	CD73 plays an important part in the adenosinergic pathway governing the conversion of AMP into adenosine. This activity impacts the signaling and function of the adenosine receptors on immune and non-immune cells. Another important pathway is the angiogenesis process where CD73 contributes by affecting endothelial cell migration and blood vessel development. Relationships with key proteins such as adenosine A2 receptors help facilitate these critical pathway activities.
Associated diseases and disorders	CD73's involvement with immunosuppression and tumor progression becomes significant. Elevated levels of CD73 are frequently observed in some cancers where it aids in creating a favorable tumor microenvironment through increased adenosine production. This situation assists tumor cells in evading immune detection. Additionally CD73 is linked to autoimmune diseases like multiple sclerosis where it contributes to modulated lymphocyte activity. Connections with proteins such as TGF-beta further illustrate its role in disease-related pathways creating potential targets for therapeutic interventions through the use of anti-CD73 antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

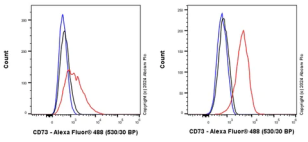
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

13 product images

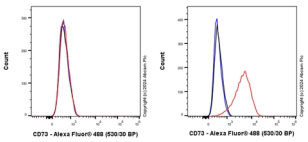


Flow Cytometry - Anti-CD73 antibody [RM2050] (ab317462)

Flow cytometric analysis of HeLa(human cervical adenocarcinoma epithelial cell, Left) / U-87 MG(human glioblastoma-astrocytoma epithelial cell, Right) cells labelling CD73 with ab317462 at 1/5000 dilution (0.01 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Low expression: HeLa (PMID: 8566797)
Gated on viable cell.

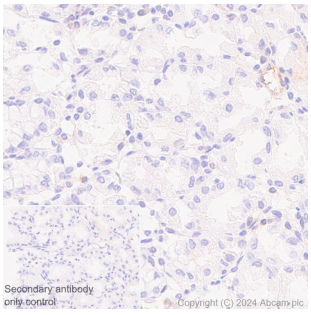


Flow Cytometry - Anti-CD73 antibody [RM2050] (ab317462)

Flow cytometric analysis of CD73 knockout A431(huam CD73 knock out human epidermoid carcinoma epithelial cell, Left) / Parental A431(Right) cells labelling CD73 with ab317462 at 1/500 dilution (0.1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Gated on viable cell.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD73 antibody [RM2050] (ab317462)

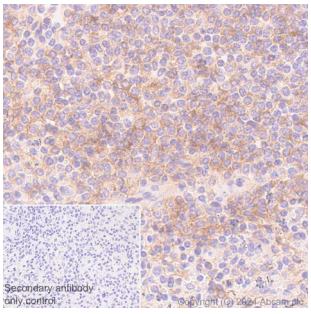
Immunohistochemical analysis of paraffin-embedded Human stomach tissue labeling CD73 with ab317462 at 1/100 (4.97 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Low expression: Weakly positive staining on blood vessels of human stomach. The section was incubated with ab317462 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD73 antibody [RM2050] (ab317462)

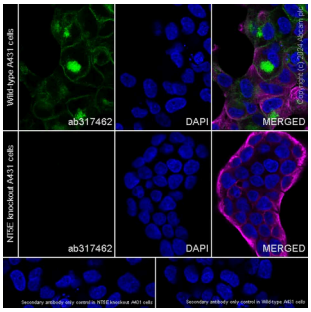
Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling CD73 with ab317462 at 1/100 (4.97 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Positive staining on human spleen. The section was incubated with ab317462 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins



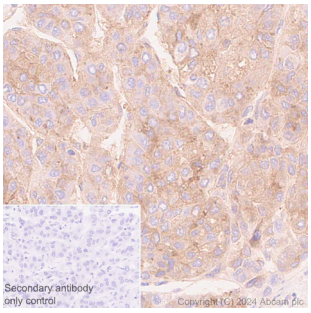
Immunocytochemistry/ Immunofluorescence - Anti-CD73 antibody [RM2050] (ab317462)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NT5E KO A431(human epidermoid carcinoma epithelial cell) cells labelling CD73 with ab317462 at 1/500 (0.994 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/mL dilution (Green).

Confocal image showing membrane and cytoplasmic staining in parental A431 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labeled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD73 antibody [RM2050] (ab317462)

Immunohistochemical analysis of paraffin-embedded Human liver cancer tissue labeling CD73 with ab317462 at 1/100 (4.97 ug/ml) dilution, followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

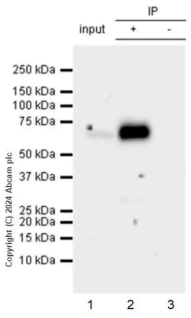
Positive staining on human liver cancer (PMID: 30971294). The section was incubated with ab317462 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument

Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

---



Immunoprecipitation - Anti-CD73 antibody [RM2050] (ab317462)

CD73 was immunoprecipitated from 0.35 mg U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate with ab317462 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab317462 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 2: ab317462 IP in U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab317462 in U-87 MG whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-CD73 antibody [RM2050] (ab317462) at 1/30 dilution

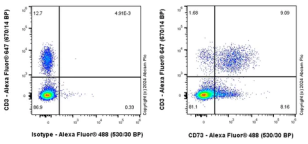
Lane 1:
U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate

Lane 2:
ab317462 IP in U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 32s

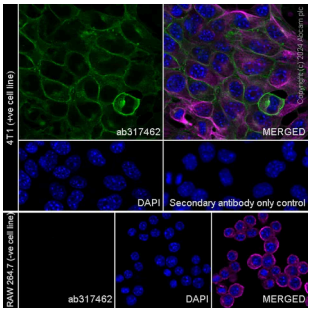


Flow Cytometry - Anti-CD73 antibody [RM2050] (ab317462)

Flow cytometric analysis of Mouse spleen cells labelling CD73 with ab317462 at 1/50 dilution (1 ug)/Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Mouse spleen cell are co-stained with CD3 conjugated Alexa Fluor®647.
Gated on viable cell.



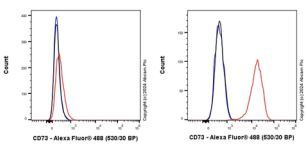
Immunocytochemistry/ Immunofluorescence - Anti-CD73 antibody [RM2050] (ab317462)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized 4T1 (mouse mammary gland carcinoma epithelial cell) cells labelling CD73 with ab317462 at 1/500 (0.994 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/mL dilution (Green).

Confocal image showing membrane and cytoplasmic staining in 4T1 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labeled with DAPI (shown in blue).
Negative control: RAW 264.7 (PMID: 28060378)
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Magenta). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution.

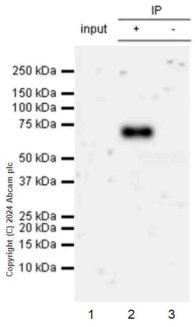


Flow Cytometry - Anti-CD73 antibody [RM2050] (ab317462)

Flow cytometric analysis of RAW 264.7(mouse Abelson murine leukemia virus-induced tumor macrophage, Left) / 4T1 (mouse mammary gland carcinoma epithelial cell, Right) cells labelling CD73 with ab317462 at 1/50 dilution (1 ug)/Red compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.

Negative control: RAW 264.7(PMID:28060378)
Gated on viable cell.



Immunoprecipitation - Anti-CD73 antibody [RM2050] (ab317462)

CD73 was immunoprecipitated from 0.35 mg 4T1 (mouse mammary gland carcinoma epithelial cell) whole cell lysate with ab317462 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab317462 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: 4T1 (mouse mammary gland carcinoma epithelial cell) whole cell lysate
Lane 2: ab317462 IP in 4T1 (mouse mammary gland carcinoma epithelial cell) whole cell lysate
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab317462 in 4T1 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-CD73 antibody [RM2050] (ab317462) at 1/30 dilution

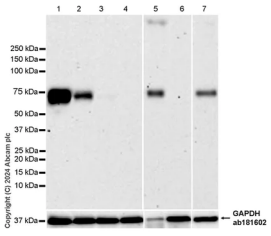
Lane 1:
4T1 (mouse mammary gland carcinoma epithelial cell) whole cell lysate

Lane 2:
ab317462 IP in 4T1 (mouse mammary gland carcinoma epithelial cell) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 180s



Western blot - Anti-CD73 antibody [RM2050] (ab317462)

Negative control: HeLa and Raji (PMID: 8566797)

In Western blot, ab317462 was shown to bind specifically to CD73. A band was observed at 75 kDa in wild-type A431 cell lysates (lane 5) whereas loss of signal was observed in the CD73 knockout cell line (lane 6, ab261895/knockout cell lysate ab261704).

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

All lanes:
Western blot - Anti-CD73 antibody [RM2050] (ab317462) at 1/1000 dilution

Lane 1:
U-87 MG (human glioblastoma-astrocytoma epithelial cell) whole cell lysate at 40 µg

Lane 2:
A375 (human malignant melanoma epithelial cell) whole cell lysate at 40 µg

Lane 3:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 40 µg

Lane 4:
Raji (human Burkitts lymphoma B lymphocyte) whole cell lysate at 40 µg

Lane 5:
Wild-type A431 (human epidermoid carcinoma epithelial cell) whole cell lysate at 40 µg

Lane 6:
CD73 knockout A431 whole cell lysate at 40 µg

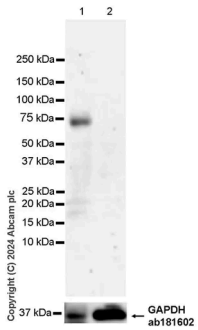
Lane 7:
Human spleen tissue lysate at 40 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 75 kDa, 36 kDa

Exposure time: 180s



Western blot - Anti-CD73 antibody [RM2050] (ab317462)

Negative control: RAW264.7 (PMID:28060378)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

All lanes:
Western blot - Anti-CD73 antibody [RM2050] (ab317462) at 1/1000 dilution

Lane 1:
4T1 (mouse mammary gland carcinoma epithelial cell) whole cell lysate at 40 µg

Lane 2:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 40 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 75 kDa, 36 kDa

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.