Human 5'-Nucleotidase/CD73 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG

Catalog Number: AF5795

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human 5'-Nucleotidase/CD73 in direct ELISAs and Western blots. In direct ELISAs, approximately 50% cross-reactivity with recombinant mouse CD73 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human 5'-Nucleotidase/CD73 Trp27-Lys547 Accession # AAH65937
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Dual RNAscope ISH-IHC Compatible	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human colon cancer
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	1-15 µg/mL	See Below
Simple Western	10 µg/mL	See Below

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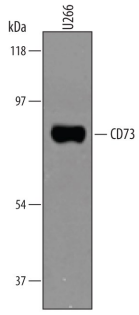
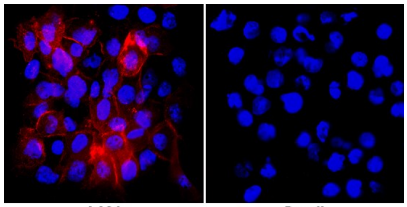
DATA

Western Blot



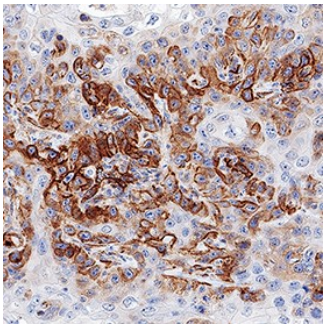
Detection of Human 5'-Nucleotidase/CD73 by Western Blot. Western blot shows lysates of U266 human myeloma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human 5'-Nucleotidase/CD73 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5795) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # Catalog # HAF016). A specific band was detected for 5'-Nucleotidase/CD73 at approximately 70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunocytochemistry



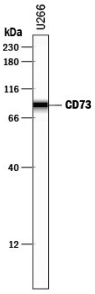
5'-Nucleotidase/CD73 in A431 Human Cell Line. 5'-Nucleotidase/CD73 was detected in immersion fixed A431 human epithelial carcinoma cell line using Sheep Anti-Human 5'-Nucleotidase/CD73 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5795) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to plasma membrane. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



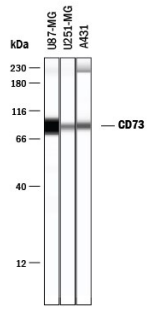
5'-Nucleotidase/CD73 in Human Cervical Cancer Tissue. 5'-Nucleotidase/CD73 was detected in immersion fixed paraffin-embedded sections of human cervical cancer tissue using Sheep Anti-Human 5'-Nucleotidase/CD73 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5795) at 1 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membranes in cancer cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



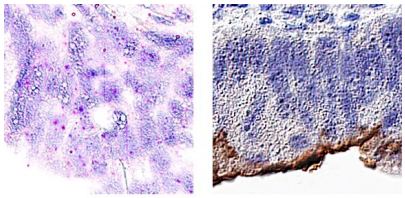
Detection of Human 5'-Nucleotidase/CD73 by Simple Western™. Simple Western lane view shows lysates of U266 human myeloma cell line, loaded at 0.5 mg/mL. A specific band was detected for 5'-Nucleotidase/CD73 at approximately 86 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human 5'-Nucleotidase/CD73 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5795) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Simple Western



Detection of Human 5'-Nucleotidase/CD73 by Simple Western™. Simple Western lane view shows lysates of U-87 MG human glioblastoma/astrocytoma cell line, U-251 MG human glioblastoma cell line, and A431 human epithelial carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for 5'-Nucleotidase/CD73 at approximately 86 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human 5'-Nucleotidase/CD73 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5795) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.

In-situ Hybridization



Detection of 5'-Nucleotidase/CD73 in Human Colon Cancer. Formalin-fixed paraffin-embedded tissue sections of human colon cancer were probed for NT5E mRNA (ACD RNAScope Probe, catalog #437931; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using sheep anti-human NT5E polyclonal antibody (R&D Systems catalog # Catalog # AF5795) at 1ug/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-sheep IgG VisUCyte HRP Polymer Antibody (Catalog # Catalog # VC006) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to glandular cells.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

5'-Nucleotidase (also [ecto]-5'-nucleotidase/5'-NT, designated CD73) is a variably glycosylated, 69-73 kDa member of the 5'-Nucleotidase family of enzymes. It is expressed on multiple cell types, including vascular endothelium, transitional and nonkeratinized epithelium, cardiomyocytes, small intestine epithelium, $FoxP3^+$ Treg lymphocytes, FDCs and B cells. 5'-Nucleotidase hydrolyzes AMP to adenosine and phosphate. This creates diffusible nucleosides necessary for cell homeostasis, and a ligand for cell membrane adenosine receptors. Mature human 5'-Nucleotidase is a 523 amino acid (aa) GPI-linked protein (aa 27-549). It contains a large Zn-dependent nucleotidase catalytic region (aa 28-532) and a C-terminal substrate binding site (aa 500-506). On the cell surface it exists as a disulfide-linked homodimer. Two splice variants are reported. One shows a deletion of aa 405-454, and a second possesses a 12 aa substitution for aa 253-574. Over aa 1-511, human 5'-Nucleotidase shares 88% aa identity with both mouse and rat 5'-Nucleotidase.

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