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Anti-ADA Antibody Picoband™ (monoclonal, 6D4)

Catalog # ABO14798

Product Information

Application	WB, IHC, FC
Primary Accession	P00813
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-ADA Antibody Picoband™ (monoclonal, 6D4) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human.

Additional Information

Gene ID	100
Other Names	Adenosine deaminase, 3.5.4.4, Adenosine aminohydrolase, ADA, ADA1
Calculated MW	40764
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Cell membrane
Tissue Specificity	Found in all tissues, occurs in large amounts in T-lymphocytes (PubMed:20959412). Expressed at the time of weaning in gastrointestinal tissues.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Clone Names	Clone: 6D4
Immunogen	E. coli-derived human ADA recombinant protein (Position: Q135-L363). Human ADA shares 82.5% and 82.9% amino acid (aa) sequence identity with mouse and rat ADA, respectively.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

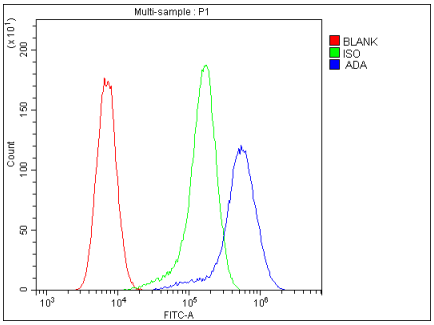
Name	ADA
Synonyms	ADA1
Function	Catalyzes the hydrolytic deamination of adenosine and 2- deoxyadenosine (PubMed:16670267, PubMed:23193172, PubMed:26166670, PubMed:8452534, PubMed:9361033). Plays an important role in purine metabolism and in adenosine homeostasis. Modulates signaling by extracellular adenosine, and so contributes indirectly to cellular signaling events. Acts as a positive regulator of T-cell coactivation, by binding DPP4 (PubMed:20959412). Its interaction with DPP4 regulates lymphocyte-epithelial cell adhesion (PubMed:11772392). Enhances dendritic cell immunogenicity by affecting dendritic cell costimulatory molecule expression and cytokines and chemokines secretion (By similarity). Enhances CD4+ T-cell differentiation and proliferation (PubMed:20959412). Acts as a positive modulator of adenosine receptors ADORA1 and ADORA2A, by enhancing their ligand affinity via conformational change (PubMed:23193172). Stimulates plasminogen activation (PubMed:15016824). Plays a role in male fertility (PubMed:21919946, PubMed:26166670). Plays a protective role in early postimplantation embryonic development (By similarity). Also responsible for the deamination of cordycepin (3'-deoxyadenosine), a fungal natural product that shows antitumor, antibacterial, antifungal, antivirus, and immune regulation properties (PubMed:26038697).
Cellular Location	Cell membrane; Peripheral membrane protein; Extracellular side. Cell junction. Cytoplasmic vesicle lumen {ECO:0000250|UniProtKB:P03958}. Cytoplasm. Lysosome. Note=Colocalized with DPP4 at the cell surface.
Tissue Location	Found in all tissues, occurs in large amounts in T- lymphocytes (PubMed:20959412). Expressed at the time of weaning in gastrointestinal tissues.

Background

Adenosine Deaminase (also known as adenosine aminohydrolase, or ADA) is an enzyme involved in purine metabolism. Primarily, ADA in humans is involved in the development and maintenance of the immune system. However, ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance. It has also been proposed that ADA, in addition to adenosine breakdown, stimulates release of excitatory amino acids and is necessary to the coupling of A1 adenosine receptors and heterotrimeric G proteins. Adenosine deaminase deficiency leads to pulmonary fibrosis, suggesting that chronic exposure to high levels of adenosine can exacerbate inflammation responses rather than suppressing them. It has also been recognized that adenosine deaminase protein and activity is upregulated in mouse hearts that overexpress HIF-1 alpha, which in part explains the attenuated levels of adenosine in HIF-1 alpha expressing hearts during ischemic stress.

Images

Figure 7. Flow Cytometry analysis of U20S cells using anti-ADA antibody (M00866).
Overlay histogram showing U20S cells stained with M00866 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-ADA Antibody (M00866,1 µg/1x10^6 cells) for 30 min



at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

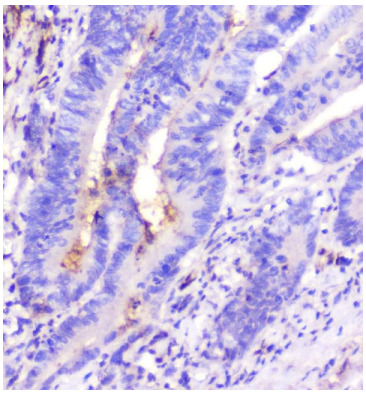


Figure 4. IHC analysis of ADA using anti-ADA antibody (M00866).
ADA was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-ADA Antibody (M00866) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

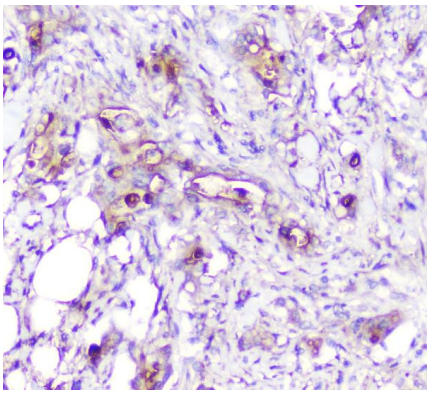


Figure 5. IHC analysis of ADA using anti-ADA antibody (M00866).
ADA was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-ADA Antibody (M00866) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

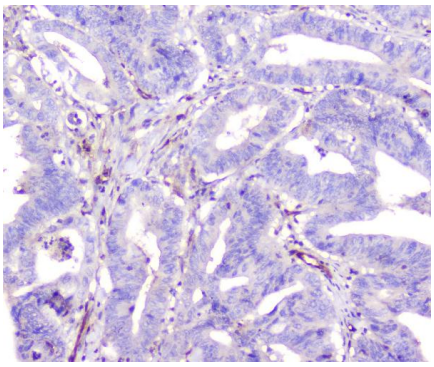
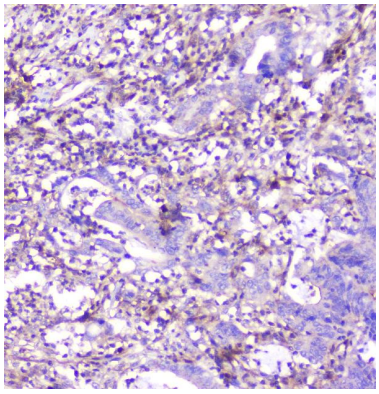


Figure 2. IHC analysis of ADA using anti-ADA antibody (M00866).
ADA was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-ADA Antibody (M00866) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Figure 3. IHC analysis of ADA using anti-ADA antibody (M00866).
ADA was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was



then incubated with 1 µg/ml mouse anti-ADA Antibody (M00866) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

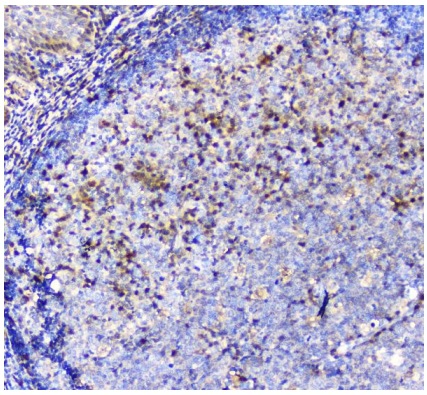


Figure 6. IHC analysis of ADA using anti-ADA antibody (M00866).
ADA was detected in paraffin-embedded section of human tonsil tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-ADA Antibody (M00866) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

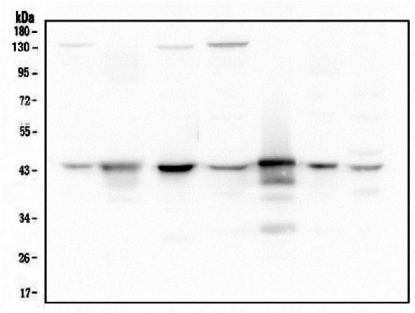


Figure 1. Western blot analysis of ADA using anti-ADA antibody (M00866).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Hela whole cell lysates,
Lane 2: human placenta tissue lysates,
Lane 3: human A549 whole cell lysates,
Lane 4: human MCF-7 whole cell lysates,
Lane 5: human U-937 whole cell lysates,
Lane 6: human U20S whole cell lysates,
Lane 7: human Caco-2 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-ADA antigen affinity purified monoclonal antibody (Catalog # M00866) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system.

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