

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

Catalog Number: M00866

About ADA

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.

Overview

Product Name	Anti-ADA Antibody Picoband® (monoclonal, 6D4)
Reactive Species	Human
Description	Boster Bio Anti-ADA Antibody Picoband® (monoclonal, 6D4) catalog # M00866. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	Flow Cytometry, IHC, WB
Clonality	Monoclonal 6D4
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
Storage Instructions	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.
Host	Mouse
Uniprot ID	P00813

Technical Details

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.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2b

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml</p> <p>Flow Cytometry (Fixed), 1-3ug/1x10⁶ cells</p>

Anti-ADA Antibody Picoband® (monoclonal, 6D4) (M00866) Images

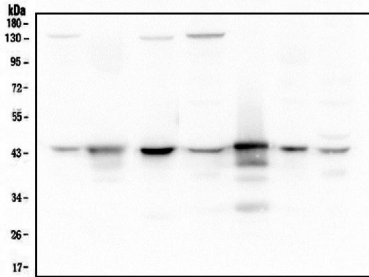


Figure 1. Western blot analysis of ADA using anti-ADA antibody (M00866).

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 ug/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.

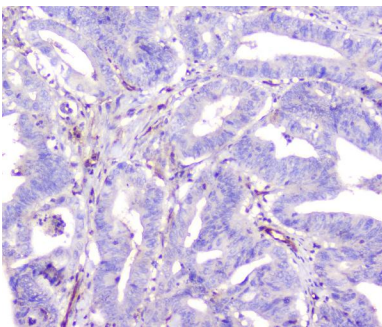


Figure 2. IHC analysis of ADA using anti-ADA antibody (M00866).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

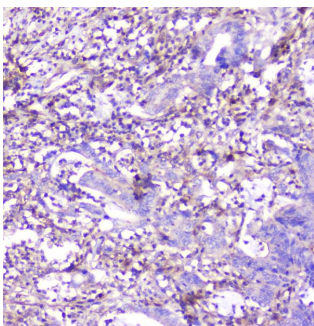
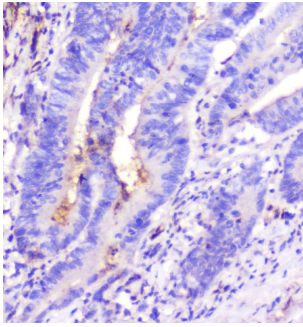


Figure 3. IHC analysis of ADA using anti-ADA antibody (M00866).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

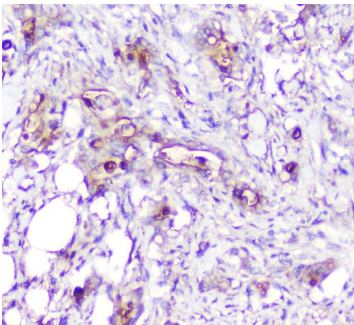


Figure 5. IHC analysis of ADA using anti-ADA antibody (M00866).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

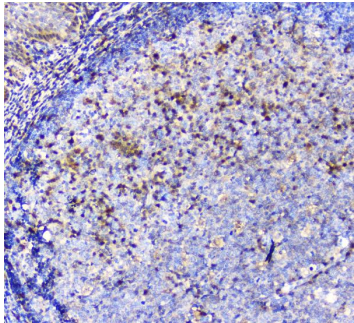


Figure 6. IHC analysis of ADA using anti-ADA antibody (M00866).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

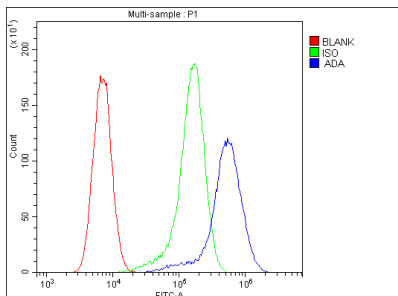


Figure 7. Flow Cytometry analysis of U20S cells using anti-ADA antibody (M00866).

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, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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