

Anti-ADA Antibody Picoband™

Catalog Number: PB9914

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™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-ADA Antibody Picoband™ catalog # PB9914. Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.01mg 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	Rabbit
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.
Predicted Reactive Species	Bovine, Canine, Monkey
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, By Heat Flow Cytometry(Fixed), 1-3 ug/1x10⁶ cells, Human

Anti-ADA Antibody Picoband™ (PB9914) Images

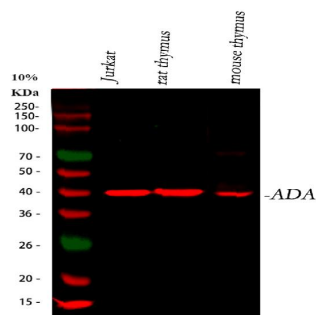


Figure 1. Western blot analysis of ADA using anti-ADA antibody (PB9914). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Jurkat whole cell lysates,
Lane 2: rat thymus tissue lysates,
Lane 3: mouse thymus tissue lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-ADA antigen affinity purified polyclonal antibody (Catalog # PB9914) 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-rabbit IgG-DyLight 647 secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. A specific band was detected for ADA at approximately 41 kDa. The expected band size for ADA is at 41 kDa.

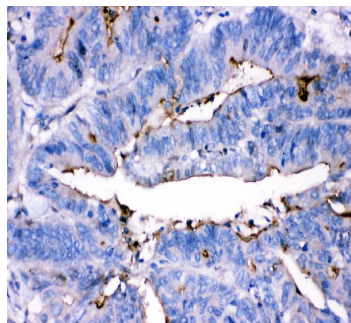


Figure 2. IHC analysis of ADA using anti-ADA antibody (PB9914).

ADA was detected in a paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-ADA Antibody (PB9914) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

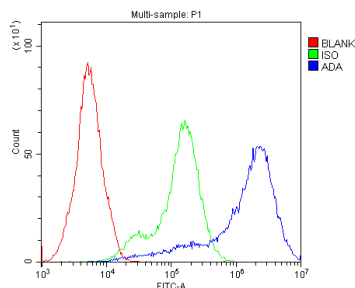


Figure 3. Flow Cytometry analysis of Jurkat cells using anti-ADA antibody (PB9914).

Overlay histogram showing Jurkat cells stained with PB9914 (Blue line). The cells were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-ADA Antibody (PB9914, 1 ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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