BOSTER BIOLOGICAL TECHNOLOGY
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Anti-ADO Antibody Picoband®

Catalog Number: PB10032

About ADO

Human thiol dioxygenases include cysteine dioxygenase (CDO) and cysteamine (2-aminoethanethiol) dioxygenase (ADO). CDO adds 2 oxygen atoms to free cysteine, whereas ADO adds 2 oxygen atoms to free cysteamine to form hypotaurine. It is demonstrated that mouse Ado has strong and specific dioxygenase activity in vitro towards cysteamine but not cysteine. Recombinant Ado was shown to bind iron. Overexpression of Ado in HepG2/C3A cells increased the production of hypotaurine from cysteamine. Similar results were found with human ADO. When endogenous expression of ADO was reduced by RNA-mediated interference, hypotaurine production decreased. It is also noted that the demonstration of high levels of ADO in brain challenges the previous assumption that most of the taurine in the brain is a consequence of CDO activity.

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Overview

Product Name	Anti-ADO Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-ADO Antibody Picoband® catalog # PB10032. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat. 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, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains antibody formulated with stabilizing components, 0.9 mg NaCl, 0.2 mg Na2HPO4, and 0.05 mg NaN3. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
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	Q96SZ5

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antibody and ELISA experts

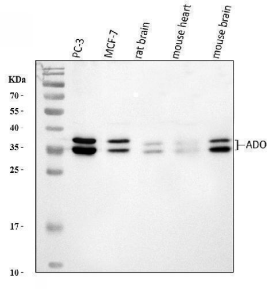
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Technical Details

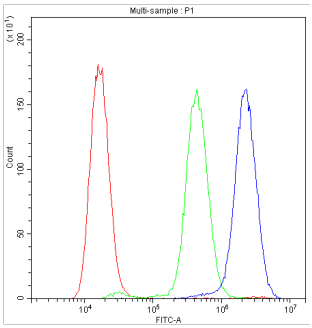
Immunogen	E. coli-derived human ADO recombinant protein (Position: E49-E261). Human ADO shares 90.1% amino acid (aa) sequence identity with mouse ADO.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
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	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Mouse, Rat Immunocytochemistry/Immunofluorescence, 2ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10^6 cells, Human Note: For flow cytometry (FCM), suitable for fixed cells only. Not suitable for FACS or surface flow cytometry using live cells.

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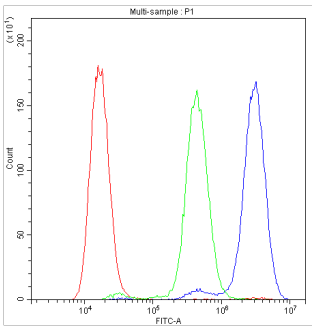
Anti-ADO Antibody Picoband® (PB10032) Images



Western blot analysis of ADO using anti-ADO antibody (PB10032). 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 PC-3 whole cell lysates, Lane 2: human MCF-7 whole cell lysates, Lane 3: rat brain tissue lysates, Lane 4: mouse heart tissue lysates, Lane 5: mouse brain 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-ADO antigen affinity purified polyclonal antibody (Catalog # PB10032) 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-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for ADO at approximately 28, 30 kDa. The expected band size for ADO is at 30 kDa.



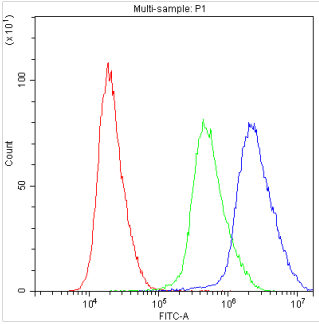
Flow Cytometry analysis of A549 cells using anti-ADO antibody (PB10032). Overlay histogram showing A549 cells stained with PB10032 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ADO Antibody (PB10032,1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



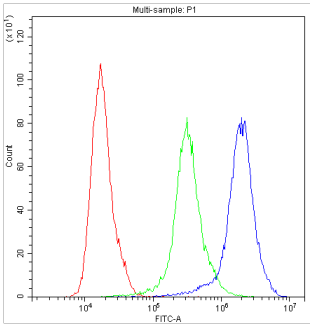
Flow Cytometry analysis of U251 cells using anti-ADO antibody (PB10032). Overlay histogram showing U251 cells stained with PB10032 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ADO Antibody (PB10032,1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

4. Flow Cytometry analysis of Hela cells using anti-ADO antibody (PB10032).Overlay histogram showing Hela cells stained with PB10032 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were

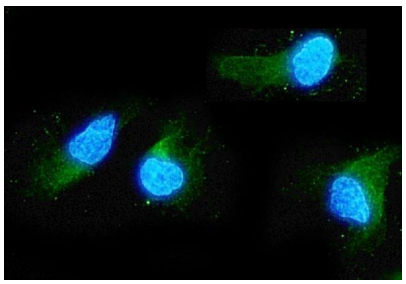
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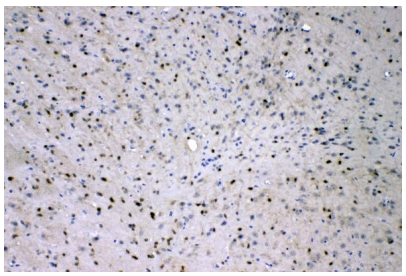
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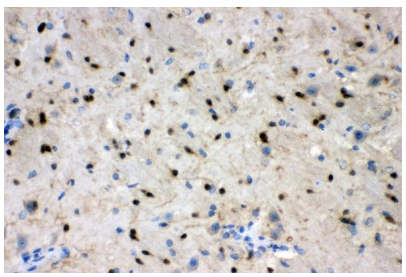
Flow Cytometry analysis of PC-3 cells using anti-ADO antibody (PB10032). Overlay histogram showing PC-3 cells stained with PB10032 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ADO Antibody (PB10032,1ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x106) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



IF analysis of ADO using anti-ADO antibody (PB10032). ADO was detected in immunocytochemical section of A549 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-ADO Antibody (PB10032) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IHC analysis of ADO using anti-ADO antibody (PB10032). ADO was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-ADO Antibody (PB10032) overnight at 4°C. Biotinylated goat anti-rabbit 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 # SA1022) with DAB as the chromogen.



IHC analysis of ADO using anti-ADO antibody (PB10032). ADO was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-ADO Antibody (PB10032) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated

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for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

Anti-ADO Antibody

For Research Use Only. Not for use in diagnostic procedures.