

Basic Information

Product Name	Anti-ADO Antibody	
Gene Name	ADO	
Source	Rabbit	
Clonality	Polyclonal	
Isotype	IgG	
Species Reactivity	human, mouse, rat	
Tested Application	WB, FCM, ICC/IF, IHC	
Contents	500 ug/ml antibody with PBS, 0.02% NaN ₃ , 1 mg/ml BSA and 50% glycerol.	
Immunogen	E. coli-derived human ADO recombinant protein (Position: E49-E261). Human ADO shares 90.1% amino acid (aa) sequence identity with mouse ADO.	
Concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	30 kDa	
Dilution Ratios	Western blot (WB): 1:500-2000 Immunohistochemistry (IHC): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow Cytometry (Fixed): 1:50-200 (Boiling the paraffin sections in 10mM citrate buffer,pH6.0,or PH8.0 EDTA repair liquid for 20 mins is required for the staining of formalin/paraffin sections.) Optimal working dilutions must be determined by end user.	

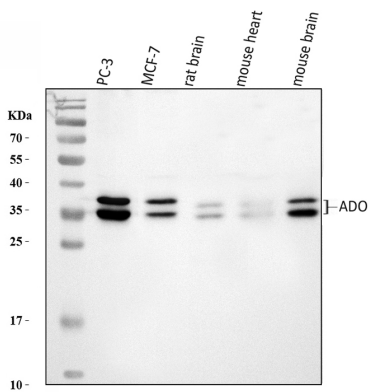
Storage

12 months from date of receipt, -20°C as supplied.

Background Information

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.

Selected Validation Data



Western blot analysis of ADO using anti-ADO antibody (PB10032). The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human PC-3 whole cell lysates,

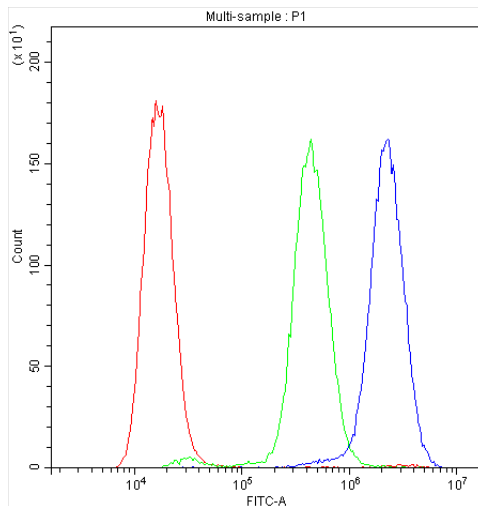
Lane 2: human MCF-7 whole cell lysates,

Lane 3: rat brain tissue lysates,

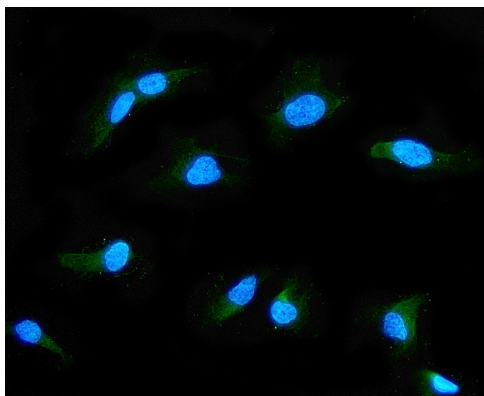
Lane 4: mouse heart tissue lysates,

Lane 5: mouse brain tissue lysates.

After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-ADO antigen affinity purified polyclonal antibody (PB10032) at a dilution of 1:1000 and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1197). A specific band was detected for ADO at approximately 30 kDa. The expected band size for ADO is at 30 kDa.

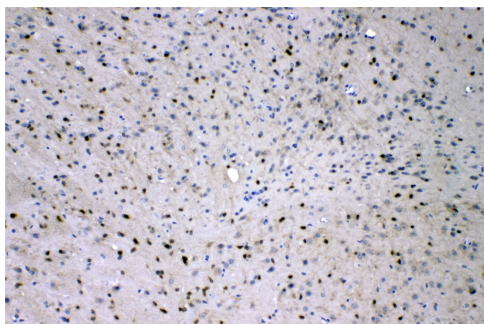


Flow Cytometry analysis of A549 cells using anti-ADO antibody (PB10032). Overlay histogram showing A549 cells stained with PB10032 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-ADO Antibody (PB10032, 1:100) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 1:100) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1:100) 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 an immunocytochemical section of A549 cells. The section was incubated with rabbit anti-ADO Antibody (PB10032) at a dilution of 1:100. DyLight®488 Conjugated Goat Anti-Rabbit IgG (Green) (Catalog # BA1127) was used as secondary antibody. The section was counterstained with DAPI (Catalog # AR1176) (Blue).



IHC analysis of ADO using anti-ADO antibody (PB10032).

ADO was detected in a paraffin-embedded section of rat brain tissue. Biotinylated goat anti-rabbit IgG was used as secondary antibody. The tissue section was incubated with rabbit anti-ADO Antibody (PB10032) at a dilution of 1:200 and developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB (Catalog # AR1027) as the chromogen.