

Basic Information

Product Name	Anti-IDO1 Antibody	
Gene Name	IDO1	
Source	Rabbit	
Isotype	IgG	
Species Reactivity	human	
Tested Application	WB, IHC, IHC-F, ICC/IF, FCM	
Contents	500 ug/ml antibody with PBS , 0.02% NaN ₃ , 1 mg BSA and 50% glycerol.	
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human IDO1 (37-69aa NDWMFIKHLPLDIESGQLRERVEKLNMLSIDH), different from the related mouse sequence by fourteen amino acids, and from the related rat sequence by seventeen amino acids.	
concentration	500 ug/ml	
Purification	Immunogen affinity purified.	
Observed MW	45KD	
Dilution Ratios	Western blot(WB): 1:500-2000 Immunohistochemistry(Paraffin-embedded Section): 1:50-400 Immunohistochemistry(Frozen Section): 1:50-400 Immunocytochemistry/Immunofluorescence (ICC/IF): 1:50-400 Flow cytometry (FCM): 1-3 µg/1x10 ⁶ cells (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. 6 months 2 to 8°C after reconstitution. Avoid repeated freezing and thawing.

Background Information

IDO1 (INDOLEAMINE 2,3-DIOXYGENASE), INDO or IDO, is an immunomodulatory enzyme produced by some alternatively activated macrophages and other immunoregulatory cells. This enzyme catalyzes the degradation of the essential amino acid L-tryptophan to N-formyl-kynurenine. By fluorescence in situ hybridization, the assignment is narrowed to chromosome 8p12-p11. INDO Interferon-gamma has an antiproliferative effect on many tumor cells and inhibits intracellular pathogens such as Toxoplasma and chlamydia, at least partly because of the induction of indoleamine 2,3-dioxygenase. During inflammation, IDO is upregulated in dendritic cells and phagocytes by proinflammatory stimuli, most notably IFNG, and the enzyme then uses superoxide as a 'cofactor' for oxidative cleavage of the indole ring of tryptophan, yielding an intermediate that deformylates to L-kynurenine.

Selected Validation Data

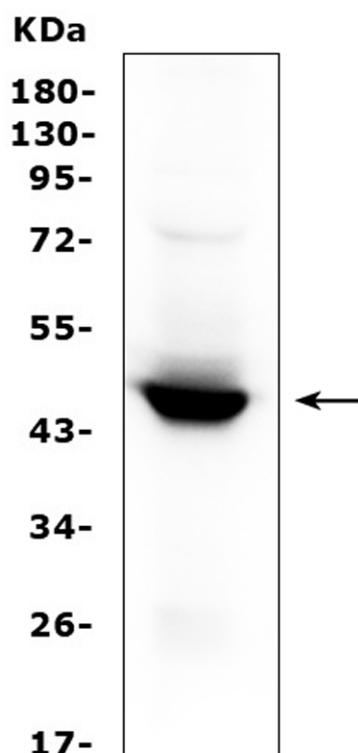


Figure 1. Western blot analysis of IDO1 using anti- IDO1 antibody (PB9603).

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 placenta tissue 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 rabbit anti- IDO1 antigen affinity purified polyclonal antibody (Catalog # PB9603) 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-rabbit 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 # EK1002) with Tanon 5200 system. A specific band was detected for IDO1 at approximately 45KD. The expected band size for IDO1 is at 45KD.

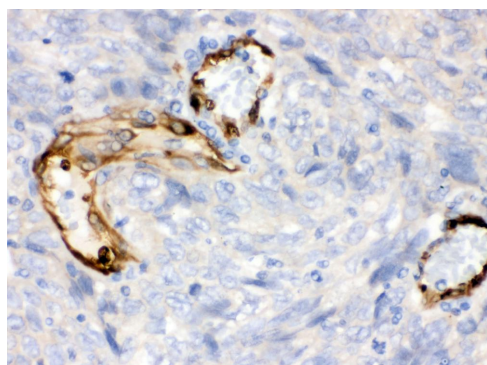


Figure 2. IHC analysis of IDO1 using anti-IDO1 antibody (PB9603).

IDO1 was detected in paraffin-embedded section of Human Lung 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 1µg/ml rabbit anti-IDO1 Antibody (PB9603) 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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

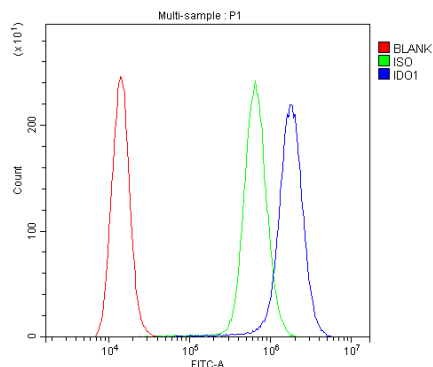


Figure 4. Flow Cytometry analysis of A431 cells using anti-IDO1 antibody (PB9603).

Overlay histogram showing A431 cells stained with PB9603 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-IDO1 Antibody (PB9603, $1\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C . DyLight488 conjugated goat anti-rabbit IgG (BA1127, $5\text{-}10\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C . Isotype control antibody (Green line) was rabbit IgG ($1\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

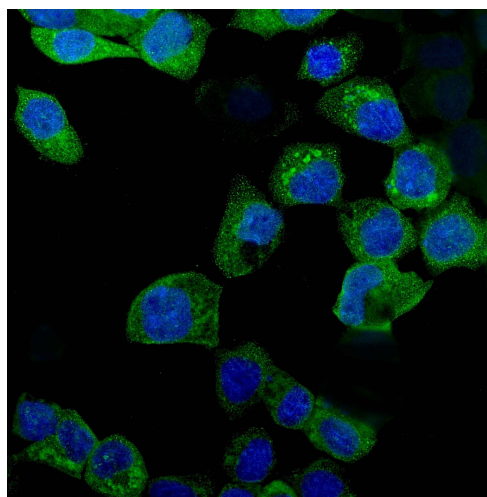


Figure 5. ICC analysis using anti-IDO1 antibody (PB9603) was detected in immersion fixed A431 cell line. Cells were stained using the DyLight488-conjugated Anti-rabbit IgG Secondary Antibody (green) (Catalog# BA1127) and counterstained with DAPI (blue).