

Anti-IDO Antibody [PD00-62]

HA721331



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|----------------------------|---|
| Product Type: | Recombinant Rabbit monoclonal IgG, primary antibodies |
| Species reactivity: | Human |
| Applications: | IHC-P, IF-Cell, WB, mIHC |
| Molecular Wt: | Predicted band size: 45 kDa |
| Clone number: | PD00-62 |

Description: Indoleamine 2,3-dioxygenase (IDO) is an IFN- γ inducible enzyme that catalyzes the degradation of the essential amino acid L-tryptophan to N-formylkynurenine. The gene encoding human IDO maps to chromosome 8p12-p11. IDO, also known as INDO, is an important modulator of immunological responses and protects allogeneic concepti from alloreactive maternal lymphocytes. IDO mediates an interesting inhibitory effect of HeLa cells co-cultured with human PBLs. The ILN-2-induced proliferation response of PBLs is diminished in the presence of HeLa cells while an IDO inhbitor negates this effect. Flow cytometric analysis indicates both mature and immature CD123-positive dendritic cells suppress T cell activity using IDO. IDO-transfected cells co-cultured with T cells reduces T cell proliferation. Additionally, adopted transfer of donor T cells reduces donor T cell numbers in IDO-transgenic mice. The pharmacological or genetic manipulation of IDO may be useful for supressing undesirable T cell response.

Immunogen: Synthetic peptide derived from the C-terminus of human IDO-1 protein.

Positive control: Human endometrial carcinoma tissue, human lymph nodes tissue, human placenta tissue, HeLa cells treated with 50 ng/mL IFN-gamma for 16 hours, A549 treated with 50ng/mL IFN-gamma for 24 hours cell lysate, human tonsils tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P14902 Human

Recommended Dilutions:

| | |
|---------|-----------------|
| IHC-P | 1:200 |
| IF-Cell | 1:100 |
| WB | 1:1,000-1:2,000 |
| mIHC | 1:1,000 |

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

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Images

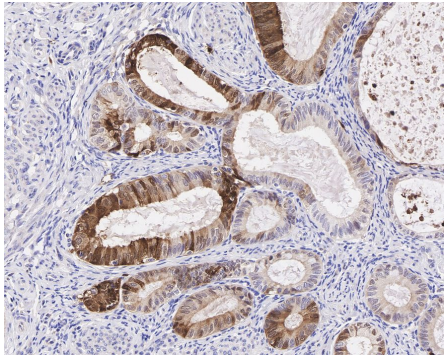


Fig1: Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue with Rabbit anti-IDO antibody (HA721331) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA721331) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

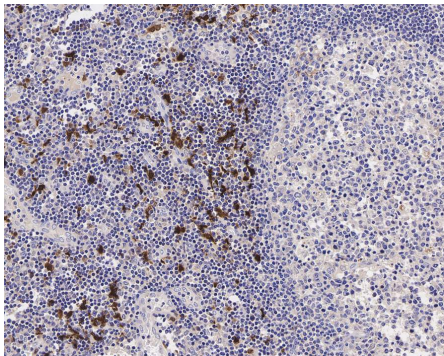


Fig2: Immunohistochemical analysis of paraffin-embedded human lymph nodes tissue with Rabbit anti-IDO antibody (HA721331) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA721331) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

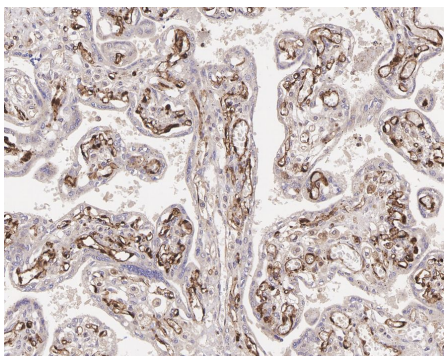
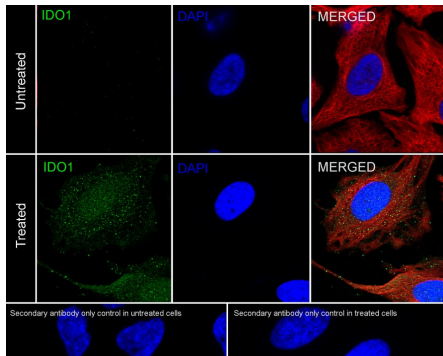


Fig3: Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-IDO antibody (HA721331) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA721331) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

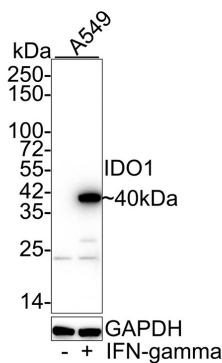
Fig4: Immunocytochemistry analysis of HeLa cells treated with or without 50 ng/mL IFN-gamma for 16 hours labeling IDO with Rabbit anti-IDO antibody (HA721331) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-IDO antibody (HA721331) at 1/100 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig5: Western blot analysis of IDO on different lysates with Rabbit anti-IDO antibody (HA721331) at 1/1,000 dilution.



Lane 1: A549 cell lysate (20 µg/Lane)

Lane 2: A549 treated with 50ng/mL IFN-gamma for 24 hours cell lysate (20 µg/Lane)

Predicted band size: 45 kDa

Observed band size: 40 kDa

Exposure time: 17 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (HA721331) at 1/1,000 dilution was used in 5% NFDN/TBST at 4 °C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

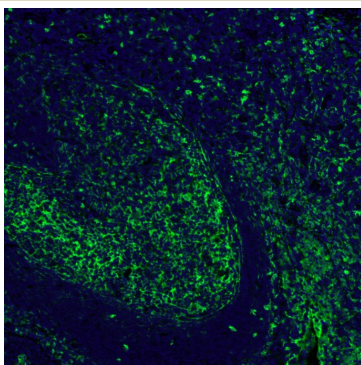


Fig6: mlHC analysis of human tonsils tissue (Formalin/PFA-fixed paraffin-embedded sections) with Rabbit anti-IDO antibody (HA721331) at 1/1,000 dilution. The immunostaining was performed with the IRISKitCmTSA Kit (900808). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95 °C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Munn D.H et al. Indoleamine 2,3 dioxygenase and metabolic control of immune responses. Trends Immunol 34:137-143 (2013).
2. Schmidt S.V et al. New insights into IDO biology in bacterial and viral infections. Front Immunol 5:384-384 (2014).

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