abcam

Product datasheet

Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] ab211017





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Overview

Product name Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374]

Description Rabbit monoclonal [EPR20374] to Indoleamine 2, 3-dioxygenase

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, ICC/IF, IP, IHC-P

Species reactivity Reacts with: Human

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Wild-type A549 Treated IFN gamma, Human ovary cancer, placenta and tonsil lysates; SK-

> OV-3 whole cell lysate; HeLa whole cell lysate treated with 50ng/ml Interferon-gamma (IFNgamma) for 16 hours. IHC-P: Human spleen, tonsil, placenta and endometrium cancer tissues. ICC/IF: HeLa cells treated with IFN-gamma (50 ng/ml) for 16 hours. Flow Cyt (intra): HeLa cells treated with IFN-gamma (50 ng/ml) for 16 hours. IP: HeLa whole cell lysate treated with 50ng/ml

IFN-gamma for 16h.

General notes This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR20374

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab211017 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	**** <u>(1)</u>	1/1000. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).
ICC/IF		1/2000.
IP		1/40.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function Catalyzes the cleavage of the pyrrol ring of tryptophan and incorporates both atoms of a molecule

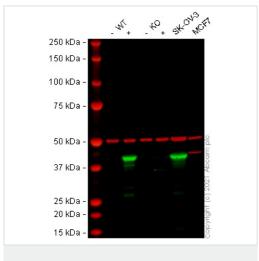
of oxygen

Pathway Amino-acid degradation; L-tryptophan degradation via kynurenine pathway; L-kynurenine from L-

tryptophan: step 1/2.

Sequence similarities Belongs to the indoleamine 2,3-dioxygenase family.

Images



Western blot - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

All lanes : Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017) at 1/1000 dilution

Lane 1 : Wild-type A549 Vehicle Control IFN gamma (0 ng/ml, 48 h) cell lysate

Lane 2: Wild-type A549 Treated IFN gamma (25 ng/ml, 48 h) cell lysate

Lane 3: IDO1 knockout A549 Vehicle Control IFN gamma (0 ng/ml, 48 h) cell lysate

Lane 4: IDO1 knockout A549 Treated IFN gamma (25 ng/ml, 48 h) cell lysate

Lane 5 : SK-OV-3 cell lysate
Lane 6 : MCF7 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 45 kDa **Observed band size:** 40 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab211017 observed at 40 kDa. Red - loading control <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab211017 was shown to react with Indoleamine 2, 3-dioxygenase in treated wild-type A549 cells in Western blot with no signal observed in treated IDO1 knockout cell line ab266949 (IDO1 knockout cell lysate ab256948). Wild-type A549 and IDO1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with ab211017 and ab7291 (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW)

preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

Secondary antibody only control Copyright (©) 2016 Abcam plo

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

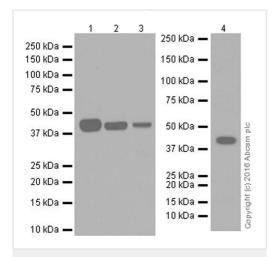
Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling Indoleamine 2, 3-dioxygenase with ab211017 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic and nuclear staining on dendritic cells of human spleen is observed (PMID: 21328335, PMID: 25271151).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

All lanes : Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017) at 1/1000 dilution

Lane 1: Human ovary cancer lysate at 20 µg

Lane 2 : Human placenta lysate at 20 µg

Lane 3: Human tonsil lysate at 20 µg

Lane 4: SK-OV-3 (Human ovarian cancer cell line) whole cell

lysate at 10 µg

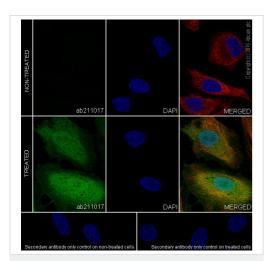
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

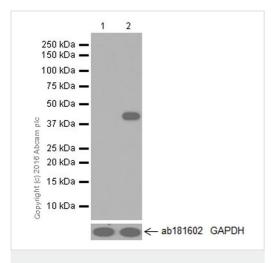
Predicted band size: 45 kDa **Observed band size:** 45 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-3: 3 minutes; Lane 4: 15 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)



Western blot - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 50ng/ml IFN- γ for 16 hours or untreated, labeling Indoleamine 2, 3-dioxygenase with ab211017 at 1/2000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

The signal increased after treatment with IFN- γ (50 ng/ml) for 16 hours on HeLa cells.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (ab150077) at 1/1000 dilution.

All lanes : Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HeLa whole cell lysate treated with 50ng/ml Interferongamma (IFN-gamma, <u>ab51240</u>) for 16 hours

Lysates/proteins at 10 µg per lane.

Secondary

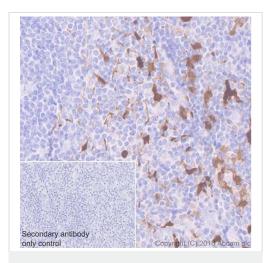
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 45 kDa **Observed band size:** 45 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The level of Indoleamine 2, 3-dioxygenase expression can be induced by IFN-y treatment (PMID 16368976).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

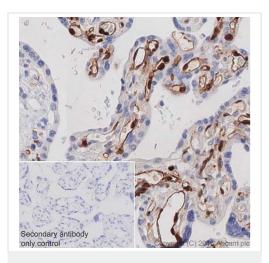
Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling Indoleamine 2, 3-dioxygenase with ab211017 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic and nuclear staining on dendritic cells of human tonsil is observed (PMID: 21328335, PMID: 25271151).

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

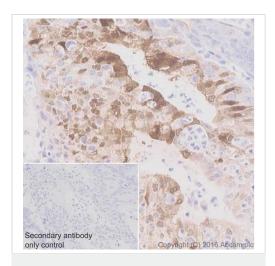
Immunohistochemical analysis of paraffin-embedded human placenta tissue labeling Indoleamine 2, 3-dioxygenase with ab211017 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Cytoplasmic and nuclear staining on endothelial cells of human placenta is observed (PMID: 21328335, PMID: 25271151).

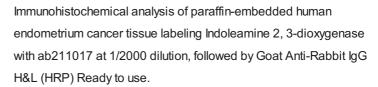
Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

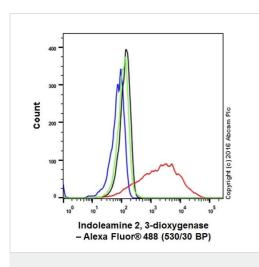


Cytoplasmic and nuclear staining on human endometrium cancer is observed (PMID: 26155395).

Counter stained with Hematoxylin.

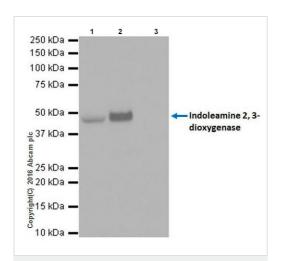
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 50ng/ml IFN-? for 16h (red) or untreated (green), labeling Indoleamine 2, 3-dioxygenase with ab211017 at 1/500 dilution compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor[®] 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Indoleamine 2, 3dioxygenase antibody [EPR20374] (ab211017)

Indoleamine 2, 3-dioxygenase was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 50ng/ml IFN- γ for 16h with ab211017 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab211017 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

Lane 1: HeLa treated with 50ng/ml IFN- γ for 16h whole cell lysate 10 μg (Input).

Lane 2: ab211017 IP in HeLa treated with 50ng/ml IFN- γ for 16h whole cell lysate.

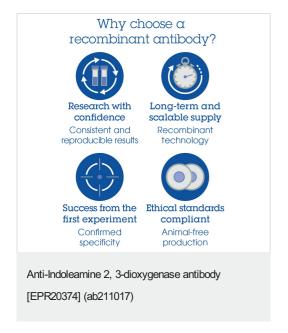
Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab211017 in HeLa treated with 50ng/ml IFN-y for 16h whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] (ab211017)

Tissue Microarrays stained for "Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374]" using "ab211017" in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The sections were pre-treated using Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0). The sections were incubated with ab211017 at +4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer).



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