# abcam

## Product datasheet

## Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free ab224263



ועלטעבע RabMAb

#### 画像数 10

#### 製品の概要

製品名 Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free

製品の詳細 Rabbit monoclonal [EPR20374] to Indoleamine 2, 3-dioxygenase - Low endotoxin, Azide free

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), ICC/IF, IP, WB, IHC-P

交差種: Human

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

> 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.

特記事項 ab224263 is the carrier-free version of ab211017.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

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

Our Low endotoxin, azide-free formats have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional

種交差性

ポジティブ・コントロール

## 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

**バッファー** pH: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** EPR20374

アイソタイプ lgG

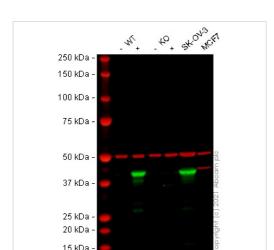
## アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab224263の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration. <b>ab199376</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

## ターゲット情報

機能	Catalyzes the cleavage of the pyrrol ring of tryptophan and incorporates both atoms of a molecule of oxygen.
パスウェイ	Amino-acid degradation; L-tryptophan degradation via kynurenine pathway; L-kynurenine from L-tryptophan: step 1/2.
配列類似性	Belongs to the indoleamine 2,3-dioxygenase family.



Western blot - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)

**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.

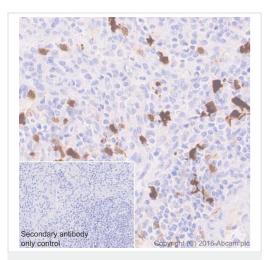
Performed under reducing conditions.

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

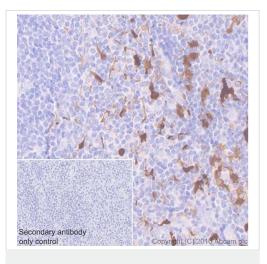
This data was developed using the same antibody clone in a different buffer formulation (ab211017).

**Lanes 1 - 6:** Merged signal (red and green). Green - <u>ab211017</u> 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 (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)

Immunohistochemical analysis of paraffin-embedded human spleen tissue labeling Indoleamine 2, 3-dioxygenase with <u>ab211017</u> 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab211017).

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

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

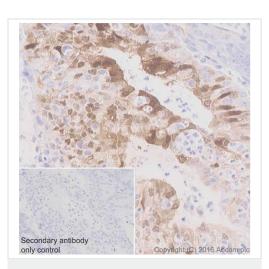
Cytoplasmic and nuclear staining on dendritic cells of human tonsil is observed (PMID: 21328335, PMID: 25271151).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab211017).

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] - Low endotoxin, Azide free (ab224263)

ab211017 DAPI MERGED

ab211017 DAPI MERGED

Immunocytochemistry/ Immunofluorescence - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)

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

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab211017).

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

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 lgG (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green).

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

The nuclear counterstain is DAPI (blue).

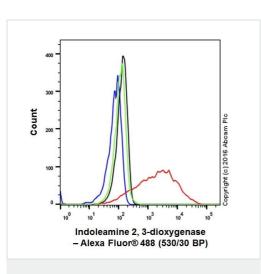
Tubulin is detected with <u>ab195889</u> (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594)) at 1/200 dilution (red).

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

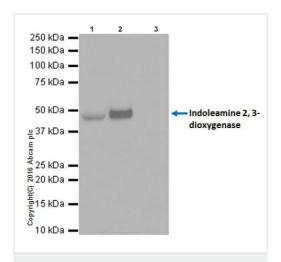
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab211017</u>).

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 <a href="mailto:ab211017">ab211017</a> at 1/500 dilution compared with a rabbit monoclonal IgG isotype control (<a href="mailto:ab172730">ab172730</a>; 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab211017).



Flow Cytometry (Intracellular) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)



Immunoprecipitation - Anti-Indoleamine 2, 3dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)

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

Western blot was performed from the immunoprecipitate using <u>ab211017</u> 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- $\gamma$  for 16h whole cell lysate 10  $\mu$ g (Input).

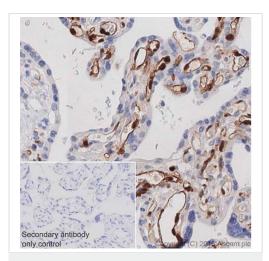
Lane 2:  $\underline{ab211017}$  IP in HeLa treated with 50ng/ml IFN- $\gamma$  for 16h whole cell lysate.

Lane 3: Rabbit monoclonal  $\lg G (\underline{ab172730})$  instead of  $\underline{ab211017}$  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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab211017).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)

Tissue Microarray (TMA) data for ab211017									
Normal fissue samples			Malignant tissue samples						
Human cardiac muscle	x	Human placenta	x (stromal cells √)	Clear cell carcinoma of human kidney	x (stromal cells ✓)	Human glioma	×		
Human cerebrum	x	Human skeletal muscle	x (stromal cells √)	Human bladder cancer	x (stromal cells ✓)	Human hepatocellular carcinoma	x		
Human colon	x (stromal cells √)	Human skin	x	Human breast carcinoma	×	Human lung carcinoma	× (stromal cells √		
Human endometrium	✓	Human spleen	✓	Human cervical carcinoma	x (stromal cells √)	Human ovarian carcinoma	x (stromal cells √		
Human kidney	x	Human stomach	x (stromal cells √)	Human colon carcinoma	x (stromal cells √)	Human poncreofic corcinoma	× (stromal cells ✓		
Human liver	x (stromal cells √)	Human testis	x	Human endometrial carcinoma	✓	Human prostatic hyperplasia	x (stromal cells √		
Human lung	x	Human thyroid	×	Human gastric adenocarcinoma	x (stromal cells ✓)	Human thyroid carcinoma	x		
Human mammary gland	*	Human tonsil	✓						
Human pancreas	x								

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Indoleamine 2, 3-dioxygenase antibody [EPR20374] - Low endotoxin, Azide free (ab224263)

This IHC data was generated using the same anti-IDO antibody clone [EPR20374] in a different buffer formulation (cat# **ab211017**).

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

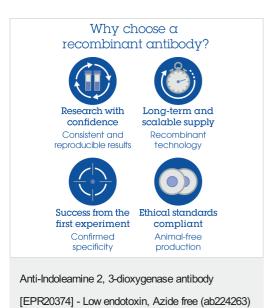
Cytoplasmic and nuclear staining on endothelial cells of human placenta is observed (PMID: 21328335, PMID: 25271151).

Counter stained with Hematoxylin.

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

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

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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