abcam

ab252438

Anti-B7H4 antibody [EPR23665-20]

Anti-B7H4 antibody [EPR23665-20] (ab252438) is a rabbit monoclonal antibody detecting B7H4 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF, mIHC**. Suitable for **Human, Mouse**.

- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR23665-20
Purification technique	Affinity purification Protein A
Concentration	0.512 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human

Dilution info	1/100
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Human
Dilution info	1/100
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Rat
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target data

See full target information VTCN1

Function	Negatively regulates T-cell-mediated immune response by inhibiting T-cell activation, proliferation, cytokine production and development of cytotoxicity. When expressed on the cell surface of tumor macrophages, plays an important role, together with regulatory T-cells (Treg), in the suppression of tumor-associated antigen-specific T-cell immunity. Involved in promoting epithelial cell transformation.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-B7H4 antibody [EPR23665-20] (ab252438) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), Multiplex IHC (mIHC) in Human, Mouse samples.

What is the molecular weight of B7H4?
Anti-B7H4 [EPR23665-20] (ab252438) specifically detects a band for B7H4 (UniProt: Q7Z7D3) at a molecular weight of 31kDa.

Other related products
We have a range of other formats of antibody clone [EPR23665-20] also available for your convenience: ab252438, Carrier free - ab272707

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	B7-H4 also known as VTCN1 or B7x is a member of the B7 family of immunoregulatory proteins. It has an approximate molecular mass of 50 kDa. B7-H4 expression is detected on the surface of various cells including B cells T cells monocytes and some cancer cells. Its expression is often inducible; immune cells can express B7-H4 in response to specific conditions or signals in the environment.
Biological function summary	B7-H4 acts as an immune checkpoint molecule that negatively regulates T cell immunity. It does not form a known complex with other proteins but instead interacts directly with T cells. By inhibiting T cell activation and proliferation B7-H4 contributes to the regulation of immune responses helping to maintain self-tolerance and prevent autoimmunity.
Pathways	B7-H4 is involved in the regulation of immune checkpoints prominently in the pathways related to immune tolerance and suppression. It plays a critical role in the pathways that modulate tumor evasion of the immune system. B7-H4 is related to other immune checkpoint proteins such as PD-L1 which are also pivotal in immune regulation and therapy targets.
Associated diseases and disorders	B7-H4 is connected to cancer progression and autoimmune conditions. Overexpression of B7-H4 in certain cancers such as ovarian and breast cancer associates with tumor immune evasion allowing tumors to grow unchecked by the immune system. Its expression links with proteins like CTLA-4 in maintaining immune suppression. B7-H4 modifications could serve as a potential target in cancer immunotherapy aiming to enhance the immune system's ability to combat malignancies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

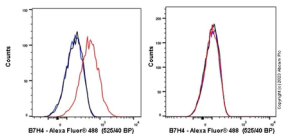
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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11 product images



Flow Cytometry - Anti-B7H4 antibody [EPR23665-20] (ab252438)

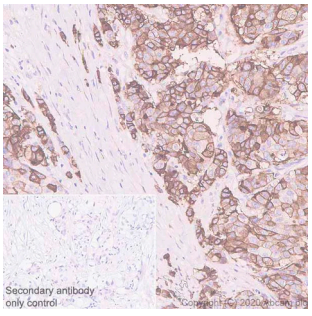
B7H4 Flow Cytometry staining using rabbit Anti-B7H4 antibody

Flow cytometry overlay histogram showing left SKBR3 positive cells and right negative HeLa stained with ab252438 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interactionfollowed by the antibody (ab252438) (1x 106 in 100µl at 10.0 µg/ml (1/50)) for 30min on ice.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



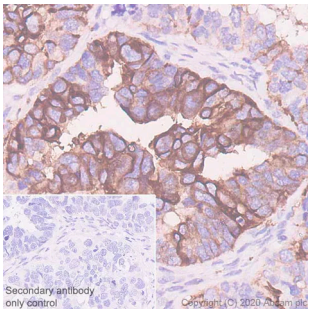
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-B7H4 antibody

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling B7H4 with ab252438 at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human breast carcinoma (PMID: 15756008). The section was incubated with ab252438 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

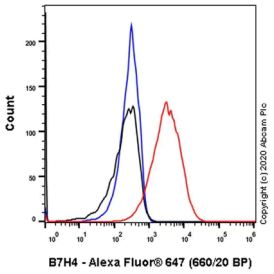


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-B7H4 antibody

Immunohistochemical analysis of paraffin-embedded Human ovarian carcinoma tissue labeling B7H4 with ab252438 at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human ovarian carcinoma (PMID: 27439899). The section was incubated with ab252438 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

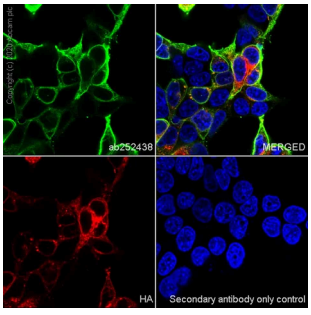
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 Flow Cytometry staining using rabbit Anti-B7H4 antibody

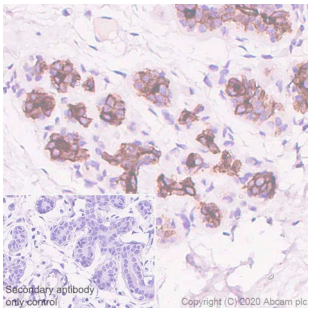
Flow cytometric analysis of SK-BR-3 (Human breast adenocarcinoma epithelial cell) cells labelling B7H4 with ab252438 at 1/500 dilution (0.1ug) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor® 647, ab150079) at 1/2000 dilution was used as the secondary antibody. Gated on viable cells.



Immunocytochemistry/ Immunofluorescence - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-B7H4 antibody

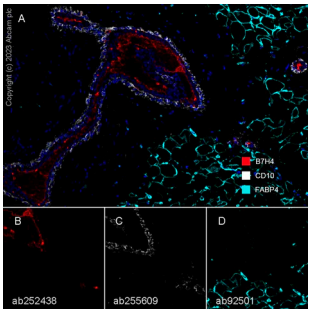
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized 293T+OE-612 cells labelling B7H4 with ab252438 at 1/50 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing cytoplasmic and membranous staining in HEK-293T cell line transfected with HA-tagged B7H4 expression vector. ab236632 Anti-HA tag rabbit monoclonal antibody was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-B7H4 antibody [EPR23665-20] (ab252438)

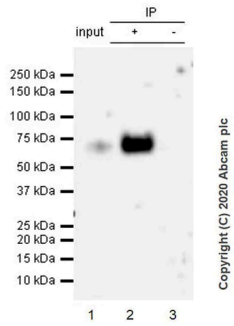
Immunohistochemical analysis of paraffin-embedded Human breast tissue labeling B7H4 with ab252438 at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human breast (PMID: 27439899). The section was incubated with ab252438 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Multiplex immunohistochemistry - Anti-B7H4 antibody [EPR23665-20] (ab252438)

Fluorescence multiplex immunohistochemical analysis of the human breast (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-B7H4 (ab252438, red; Opal™690), anti-CD10 (ab255609, gray; Opal™520) and anti-FABP4 (ab92501, cyan; Opal™570) on human breast. Panel B: anti-B7H4 stained on glandular lumens. Panel C: anti-CD10 stained on myoepithelial cells. Panel D: anti-FABP4 stained on adipocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The section was incubated in three rounds of staining: in the order of ab252438 at 1/100 dilution (4.69 µg/ml), ab255609 at 1/1000 dilution (0.615 µg/ml) and ab92501 at 1/10000 dilution (0.047 µg/ml) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain.



Immunoprecipitation - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 was immunoprecipitated from 0.35 mg SK-BR-3 (human breast adenocarcinoma epithelial cell) whole cell lysate with ab252438 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab252438 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.
Lane 1: SK-BR-3 whole cell lysate 10 ug

Lane 2: ab252438 IP in SK-BR-3 whole cell lysate 10 ug

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab252438 in SK-BR-3 whole cell lysate

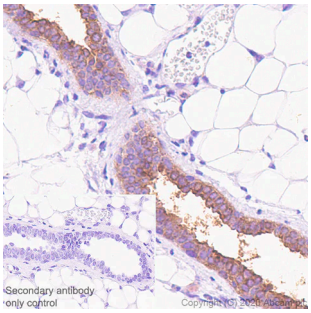
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 127 seconds

The bands at 65kDa represent glycosylated B7H4 (PMID: 15878339).

All lanes:
Immunoprecipitation - Anti-B7H4 antibody [EPR23665-20] (ab252438)

Predicted band size: 31 kDa

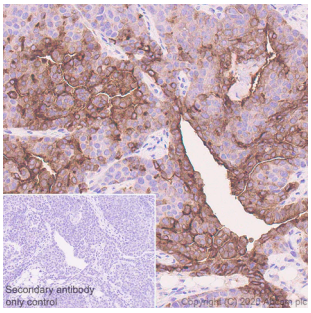


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-B7H4 antibody

Immunohistochemical analysis of paraffin-embedded mouse breast tissue labeling B7H4 with ab252438 at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).Positive staining on mouse breast. The section was incubated with ab252438 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

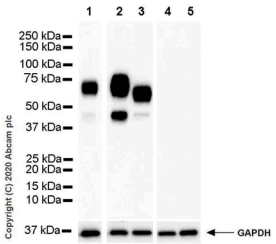


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-B7H4 antibody [EPR23665-20] (ab252438)

B7H4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-B7H4 antibody

Immunohistochemical analysis of paraffin-embedded mouse breast carcinoma tissue labeling B7H4 with ab252438 at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).Positive staining on mouse breast cancer cells. The section was incubated with ab252438 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-B7H4 antibody [EPR23665-20] (ab252438)

Blocking and diluting buffer and concentration: 5% NFDM/TBST
B7H4 is N-glycosylated protein. The level of B7H4 glycosylation varies between different cells and tissues. The bands at 65kDa and 45kDa represent glycosylated B7H4. (PMID: 15878339).

Negative control: HT-29 and HeLa. (PMID: 15878339).

Exposure time: 3 minutes

All lanes:
Western blot - Anti-B7H4 antibody [EPR23665-20] (ab252438) at 1/1000 dilution

Lane 1:
SK-BR-3 (human breast adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
MDA-MB-468 (human breast adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 3:
NIH:OVCAR-3 (human ovary adenocarcinoma epithelial cell), whole cell lysate at 10 µg

Lane 4:
HT-29 (human colorectal adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 5:
HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 31 kDa

Observed band size: 45 kDa, 65 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.