abcam

# ab269455

# Anti-B7H4 antibody [EPR20236-9]

Recombinant | RabMAb

## 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** | EPR20236-9 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.547 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

### IP

#### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/30 |
| **Notes** | - |

#### Expected

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

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/60 |
| **Notes** | - |

### Expected

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

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 |
| **Notes** | - |

## IHC-P

### Not recommended

| | |
|---|---|
| **Species** | Mouse, Human |
| **Dilution info** | - |

| | |
|---|---|
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/500 |
| **Notes** | - |

### Expected

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

## 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

**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.

## 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.

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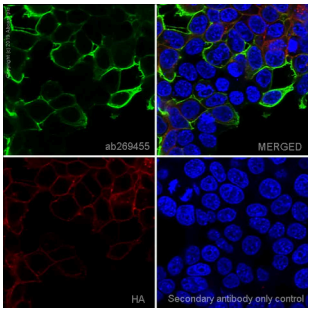
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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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## 4 product images

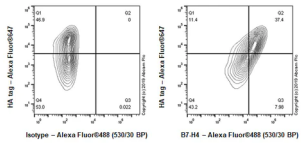



### Immunocytochemistry/ Immunofluorescence - Anti-B7H4 antibody [EPR20236-9] (ab269455)

B7H4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-B7H4 antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human embryonic kidney epithelial cell) cells labelling B7H4 with ab269455 at 1/500 dilution, followed by ab150077 AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing cytoplasmic and membranous staining in HEK-293T cell line transfected with a HA-tagged B7-H4 expression vector. Alexa Fluor® 647 anti-HA.11 Epitope Tag Antibody was used at 200 dilution to detecte the overexpressed protein (Red). The nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150077 AlexaFluo®488 Goat anti-Rabbit secondary at 1/1000 dilution.

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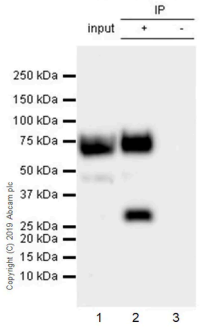


### Flow Cytometry - Anti-B7H4 antibody [EPR20236-9] (ab269455)

B7H4 Flow Cytometry staining using rabbit Anti-B7H4 antibody

Flow cytometric analysis of 2% paraformaldehyde fixed 0.1% Tween-20 permeabilized HEK-293T (human embryonic kidney epithelial cell) transfected with myc-tagged B7-H4 expression vector cells labelling B7H4 with ab269455 at 1/60 dilution (Right) compared with a Rabbit monoclonal IgG (ab172730) isotype control (Left). PMID: 23722540 and PMID: 27632942 showed that cell surface expression of B7-H4 hard to detected in vitro cultured cell lines, and all the commercial

B7H4 antibodies that recommend FC as an application use the overexpression system. Our RabMab also could recognise overexpressed B7H4.The overexpression plasmid contains the full length aa1-282 of human B7H4(Q7Z7D3) with HA tag in pcDNA3.1(+) vector.
Cells were surface stained with rabbit IgG (Left) or ab269455 (Right). Then fixed with 2% PFA followed by intracellularly stained with anti-HA tag conjugated to Alexa Fluor® 647.




## Immunoprecipitation - Anti-B7H4 antibody [EPR20236-9] (ab269455)

B7H4 was immunoprecipitated from 0.35 mg SK-BR-3 (Human breast adenocarcinoma epithelial cell) whole cell lysate with ab269455 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab269455 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 10ug.

Lane 2: ab269455 IP in SK-BR-3 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab269455 in SK-BR-3 whole cell lysate.

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

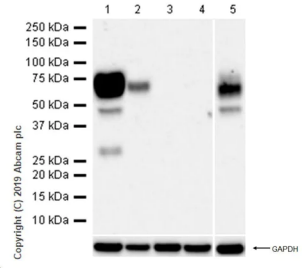
Exposure time: 30 seconds.

B7H4 is N-glycosylated protein. The level of B7H4 glycosylation varies between different cells and tissues. The band at 65kDa represents glycosylated B7H4. The band at 28kDa represents non-glycosylated B7H4 (PMID: 15878339).

All lanes:
Immunoprecipitation - Anti-B7H4 antibody [EPR20236-9] (ab269455)

Predicted band size: 31 kDa

Observed band size: 28 kDa, 65 kDa




## Western blot - Anti-B7H4 antibody [EPR20236-9] (ab269455)

B7H4 Western blot staining using rabbit Anti-B7H4 antibody

Blocking and dilution buffer: 5% NFDM/TBST.
Exposure time: 114 seconds.

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. The band at 28kDa represents non-glycosylated B7H4. (PMID: 15878339).

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

All lanes:
Western blot - Anti-B7H4 antibody [EPR20236-9] (ab269455) at 1/1000 dilution

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

Lane 2:
T47D (human ductal breast epithelial tumor epithelial cell), whole cell lysate at 20 µg

Lane 3:
HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

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

Lane 5:
Mouse uterus tissue lysate at 20 µg

Secondary

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

Predicted band size: 31 kDa

Observed band size: 28 kDa, 45 kDa, 65 kDa

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