abcam

ab300042

# Anti-VISTA antibody [EPR25068-124]

Recombinant | RabMAb | KO Validated | 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** | EPR25068-124 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.483 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## IHC-P

### Expected

| | |
|---|---|
| **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. |

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

## WB

### Tested

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

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | - |

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

## Flow Cyt

### Tested

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

### Expected

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

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

## IP

### Tested

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

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

### Expected

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

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/50 |
| **Notes** | - |

## Target data

See full target information VSIR

| | |
|---|---|
| **Function** | Immunoregulatory receptor which inhibits the T-cell response (PubMed:24691993). May promote differentiation of embryonic stem cells, by inhibiting BMP4 signaling (By similarity). May stimulate MMP14-mediated MMP2 activation (PubMed:20666777). |

## 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** | VISTA also known as V-domain Ig suppressor of T cell activation or Gi24 is a protein significant in immune regulation. It is a type I transmembrane glycoprotein with an apparent mass of approximately 55 kDa. VISTA is expressed on hematopoietic cells specifically within immune system components such as myeloid cells and T cells. Researchers have identified biotinylated chimeric proteins which facilitate the study of VISTA's expression and interactions in various immunological research models. |
| **Biological function summary** | VISTA functions in immune checkpoint regulation by modulating T cell activity. It acts as both a ligand and a receptor influencing immune responses. As part of an immune complex VISTA interacts with other checkpoint proteins to maintain immune homeostasis and prevent autoimmunity. Its role mirrors those of other B7 family members like PD-1 and CTLA-4 which are involved in negative immune regulation. |
| **Pathways** | VISTA integrates into the broader framework of immune signaling pathways. It contributes significantly to the immune checkpoint pathway similar to CTLA-4 and PD-1 which mediate immune cell proliferation and activation. By interacting with these proteins VISTA plays an important role in maintaining self-tolerance and regulating inflammatory responses functioning parallel to other proteins such as CD28 and B7-H5 in the immune checkpoint pathway. |
| **Associated diseases and disorders** | VISTA's improper regulation associates with autoimmune diseases and cancer. Its expression influences the progression of conditions like rheumatoid arthritis where it modulates immune responses and inflammation. Additionally in oncology VISTA acts as an inhibitory molecule; hence targeting it can enhance anti-tumor immunity similar to therapies targeting proteins like PD-1 and PD-L1. Understanding VISTA's intricate connections helps in developing therapies for these diseases. |

## 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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## 29 product images

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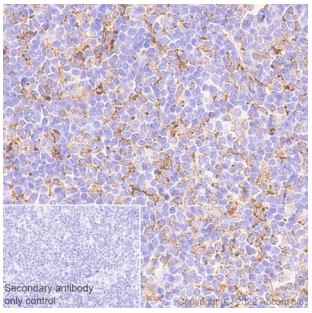

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VISTA antibody [EPR25068-124] (ab300042), expandable thumbnail

### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human spleen tissue using rabbit Anti-VISTA antibody

Immunohistochemical analysis of paraffin-embedded Human spleen tissue labeling VISTA with ab300042 at 1/100 dilution (4.92 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human spleen. The section was incubated with ab300042 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 LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

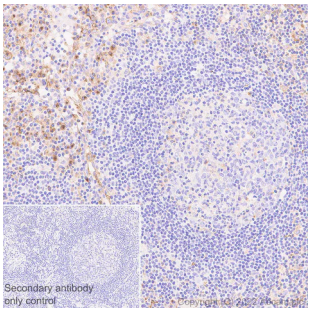
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## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human non-Hodgkin's B-Cell lymphoma tissue using rabbit Anti-VISTA antibody

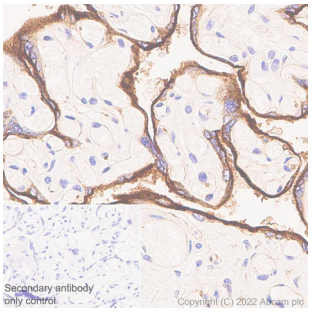
Immunohistochemical analysis of paraffin-embedded Human non-Hodgkin's B-Cell lymphoma tissue labeling VISTA with ab300042 at 1/100 dilution (4.92 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) was used. Positive staining on human non-Hodgkin's B-Cell lymphoma. The section was incubated with ab300042 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 LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human tonsil tissue using rabbit Anti-VISTA antibody

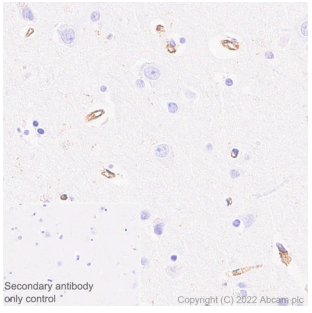
Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling VISTA with ab300042 at 1/100 dilution (4.92 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human tonsil. The section was incubated with ab300042 for 30 mins at room temperature. 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 LeicaDS9800 (Bond™ Polymer Refine Detection). 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-VISTA antibody [EPR25068-124] (ab300042)

VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human placenta tissue using rabbit Anti-VISTA antibody

Immunohistochemical analysis of paraffin-embedded Human placenta tissue labeling VISTA with ab300042 at 1/100 dilution (4.92 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human placenta. The section was incubated with ab300042 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 LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

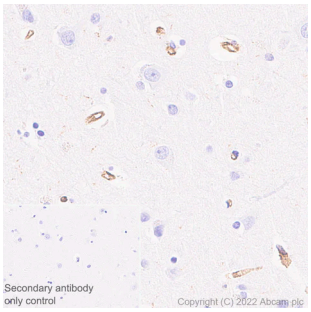



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human cerebrum tissue using rabbit Anti-VISTA antibody

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling VISTA with ab300042 at 1/100 dilution (4.92 µg/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on endothelial cells of human cerebrum. The section was

incubated with ab300042 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 LeicaDS9800 (Bond™ Polymer Refine Detection). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

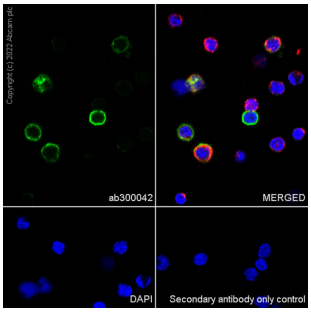



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VISTA antibody [EPR25068-124] (ab300042)

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

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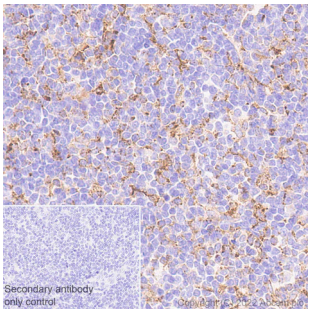
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## Immunocytochemistry/ Immunofluorescence - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-VISTA antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Human PBMC cells lebeling VISTA with ab300042 at 1/50 (9.84 µg/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 (2 µ/ml) dilution (Green). Confocal image showing cytoplasmic and membranous staining in subsets of human PBMCs. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 µg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).
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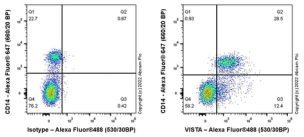



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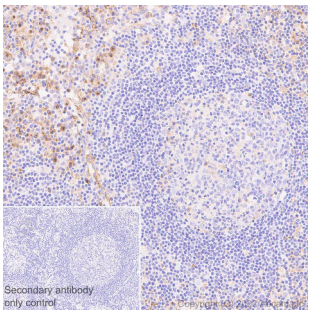
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



## Flow Cytometry - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Flow Cytometry staining using rabbit Anti-VISTA antibody

Flow cytometric analysis of Human peripheral blood mononuclear cells (PBMC) labeling VISTA with ab300042 at 1/500 dilution (0.1 ug)/ Right compared with a Rabbit monoclonal IgG (ab172730) / Left isotype control. Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody. Cells were stained with rabbit IgG or ab300042. Then stained with anti-CD14 conjugated to Alexa Fluor® 647. Gated on viable cells.

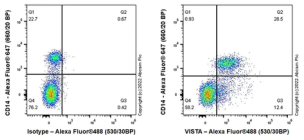



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VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-VISTA antibody

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labeling VISTA with ab300042 at 1/100 dilution (4.92 µg/ml) followed by a ready to use LeicaDS9800 (BOND™ Polymer Refine Detection). Positive staining on human tonsil. The section was incubated with ab300042 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 LeicaDS9800 (BOND™ Polymer Refine Detection).

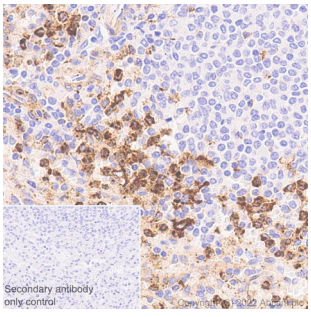
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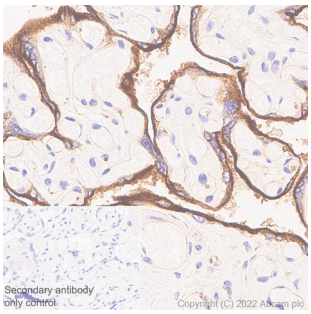

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Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

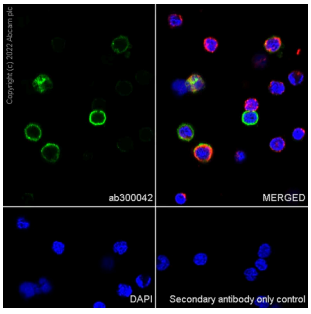



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VISTA Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-VISTA antibody

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Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042), expandable thumbnail

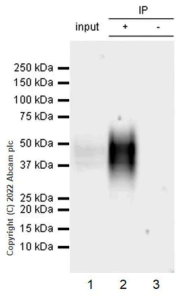
## Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA was immunoprecipitated from 0.35 mg DU-145 (Human prostate carcinoma epithelial) whole cell lysate with ab300042 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300042 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution. Lane 1: DU-145 (Human prostate carcinoma epithelial) whole cell lysate 10 µg. Lane 2: ab300042 in DU-145 whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab300042 in DU-145 whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 32.368 seconds
All lanes:
Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/30 dilution

Lane 1:
DU-145 (Human prostate carcinoma epithelial) whole cell lysate at 10 µg

Lane 2:
ab300042 in DU-145 whole cell lysate

Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab300042 in DU-145 whole cell lysate




## Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042)

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Lane 1: DU-145 (Human prostate carcinoma epithelial) whole cell lysate 10 µg

Lane 2: ab300042 in DU-145 whole cell lysate

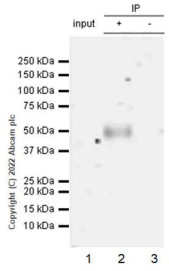
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab300042 in DU-145 whole cell lysate

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

Exposure time: 32.368 seconds

All lanes:
Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042)

Predicted band size: 34 kDa




## Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA was immunoprecipitated from 0.35 mg bEnd.3 (mouse brain endothelioma) whole cell lysate with ab300042 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300042 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: bEnd.3 (mouse brain endothelioma) whole cell lysate 10 µg

Lane 2: ab300042 in bEnd.3 whole cell lysate

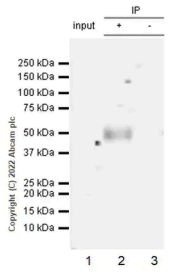
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab300042 in bEnd.3 whole cell lysate

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

Exposure time: 32 seconds

All lanes:
Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042)

Predicted band size: 34 kDa



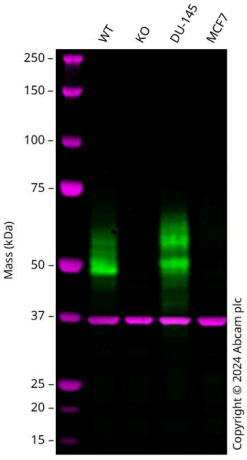

# Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA was immunoprecipitated from 0.35 mg bEnd.3 (mouse brain endothelioma) whole cell lysate with ab300042 at 1/30 dilution (2 µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab300042 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (ab131366) was used at 1/5000 dilution. Lane 1: bEnd.3 (mouse brain endothelioma) whole cell lysate 10 µg. Lane 2: ab300042 in bEnd.3 whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab300042 in bEnd.3 whole cell lysate. Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 32 seconds
All lanes:
Immunoprecipitation - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/30 dilution

Lane 1:
bEnd.3 (Mouse brain endothelioma) at 10 µg

Lane 2:
ab300042 in bEnd.3 whole cell lysate

Lane 3:
Rabbit monoclonal IgG (ab172730) instead of ab300042 in bEnd.3 whole cell lysate




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining using rabbit Anti-VISTA antibody

Western blot: Anti-VSIR antibody [EPR25068-124] (ab300042) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab300042 was shown to bind specifically to VSIR. A band was observed at 50-60 kDa in wild-type A549 cell lysates with no signal observed at this size in VSIR knockout cell line. To generate this image, wild-type and VSIR knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
Wild-type A549 cell lysate at 20 µg

Lane 2:
VSIR knockout A549 cell lysate at 20 µg

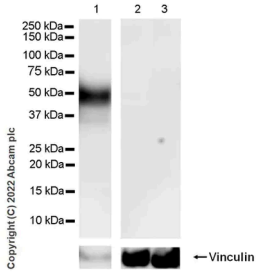
Lane 3:
DU 145 cell lysate at 20 µg

Lane 4:
MCF7 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 2:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

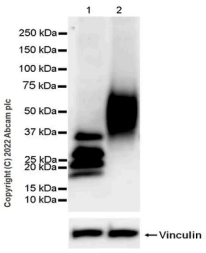
Lane 3:
HEK-293T (Human embryonic kidney) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 50 kDa

Exposure time: 37s




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration:
5% NFDM/TBST

All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
DU-145 (Human prostate carcinoma epithelial) whole cell lysate treated with Protein Deglycosylation MIX II at 15 µg

Lane 2:
Untreated DU-145 whole cell lysate at 15 µg

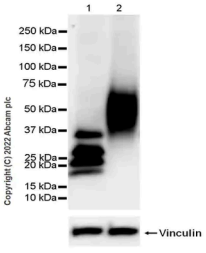
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa, 50 kDa

Exposure time: 48s




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
DU-145 (Human prostate carcinoma epithelial) whole cell lysate treated with Protein Deglycosylation MIX II at 15 µg

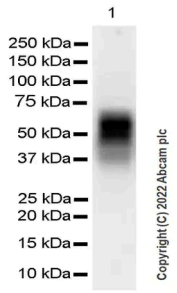
Lane 2:
Untreated DU-145 whole cell lysate at 15 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Observed band size: 34 kDa, 50 kDa

Exposure time: 48s




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining of Human heart tissue lysate using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

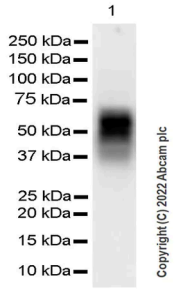
All lanes:
Human heart tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/1000 dilution

Observed band size: 50 kDa

Exposure time: 59s




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining of Human heart tissue lysate using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration:
5% NFDM/TBST

All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

All lanes:
Human heart tissue lysate at 20 µg

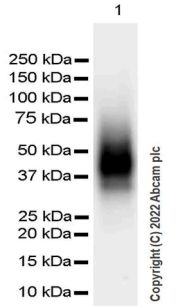
Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/1000 dilution

Predicted band size: 34 kDa

Observed band size: 50 kDa

Exposure time: 59s




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining of Human spleen tissue lysate using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration:
5% NFDM/TBST

All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

All lanes:
Human spleen tissue lysate at 20 µg

Secondary

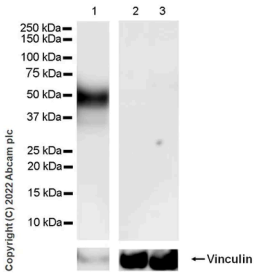
All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/1000 dilution

Predicted band size: 34 kDa

Observed band size: 37-50 kDa

Exposure time: 10s

## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)




VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration:
5% NFDM/TBST

All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 2:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
HEK-293T (human embryonic kidney) whole cell lysate at 20 µg

Secondary

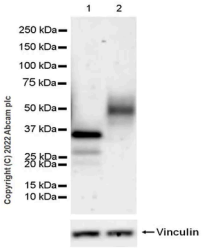
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 34 kDa

Observed band size: 50 kDa

Exposure time: 37s

## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)




VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
bEnd.3 (mouse brain endothelioma) whole cell lysate treated with Protein Deglycosylation MIX II at 15 µg

Lane 2:
Untreated bEnd.3 whole cell lysate at 15 µg

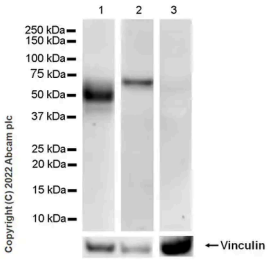
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

Predicted band size: 34 kDa

Observed band size: 34 kDa, 50 kDa

Exposure time: 125s




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
bEnd.3 (Mouse brain endothelioma) whole cell lysate at 20 µg

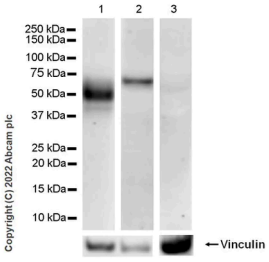
Lane 2:
EL4 (Mouse lymphoma T lymphocyte) whole cell lysate at 20 µg

Lane 3:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 50 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 50 kDa, 70 kDa




## Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042)

VISTA Western blot staining using rabbit Anti-VISTA antibody

Blocking / Diluting buffer and concentration:
5% NFDM/TBST

All lanes:
Western blot - Anti-VISTA antibody [EPR25068-124] (ab300042) at 1/1000 dilution

Lane 1:
bEnd.3 (mouse brain endothelioma) whole cell lysate at 20 µg

Lane 2:
EL4 (mouse lymphoma T lymphocyte) whole cell lysate at 20 µg

Lane 3:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 50 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 34 kDa

Observed band size: 50 kDa, 70 kDa

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