abcam

Product datasheet

Anti-HAI-1 antibody [EPR23294-49] ab272721

Recombinant RabMAb

9 Images

Overview

Product name Anti-HAI-1 antibody [EPR23294-49]

Description Rabbit monoclonal [EPR23294-49] to HAI-1

Host species Rabbit

Unsuitable for human and rat IHC-P. Specificity

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

Unsuitable for: IHC-Fr

Reacts with: Mouse, Rat, Human Species reactivity

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human and rat placenta tissue lysate. T47D whole cell lysate. Mouse large intestine, small

intestine, placenta and stomach tissue lysate. ICC/IF: T47D cells. Flow Cyt (intra): T47D cells.

IHC-P: Mouse testis and stomach tissue. IP: mouse placenta tissue.

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.

pH: 7.2 Storage buffer

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR23294-49

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab272721 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)		1/600. (0.1ug)
WB		1/1000. Detects a band of approximately 56, 66 kDa (predicted molecular weight: 58 kDa). (0.616 µg/ml).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. This antibody is not suitable for human and rat species in IHC-P application.
ICC/IF		1/50.
IP		1/30. (2µg in 0.35mg lysates)

Application notes Is unsuitable for IHC-Fr.

Target

Function Inhibitor of HGF activator. Also acts as an inhibitor of matriptase (ST14).

Sequence similarities Contains 2 BPTI/Kunitz inhibitor domains.

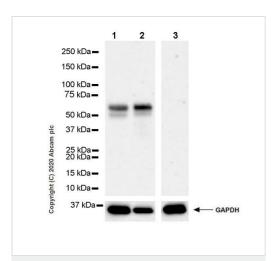
Contains 1 LDL-receptor class A domain.

Contains 1 MANSC domain.

Domain This inhibitor contains two inhibitory domains.

Cellular localization Secreted.

Images



Western blot - Anti-HAl-1 antibody [EPR23294-49] (ab272721)

All lanes : Anti-HAI-1 antibody [EPR23294-49] (ab272721) at 1/1000 dilution

Lane 1: Human placenta tissue lysate

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

Lane 3 : Daudi (human Burkitts lymphoma lymphoblast), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 58 kDa **Observed band size:** 56,66 kDa

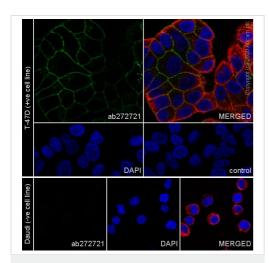
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

This blot was developed using a higher sensitivity ECL substrate.

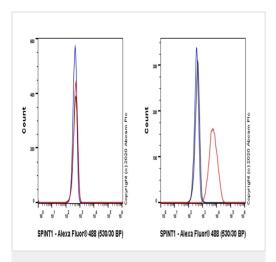
Low expression cell line: Daudi (PMID:24070417).

The molecular weight observed is consistent with what has been described in the literature (PMID:11013244).

Exposure time: 3 minutes.



Immunocytochemistry/ Immunofluorescence - Anti-HAI-1 antibody [EPR23294-49] (ab272721)



Flow Cytometry (Intracellular) - Anti-HAI-1 antibody [EPR23294-49] (ab272721)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized T-47D cells labelling HAI-1 with ab272721 at 1/50 dilution, followed by $\underline{ab150077}$ Goat Anti-Rabbit IgG H&L (Alexa Fluor $^{\circledR}$ 488) antibody at 1/1000 (2µg/ml) dilution (Green). Confocal image showing membranous staining in T-47D cell line.

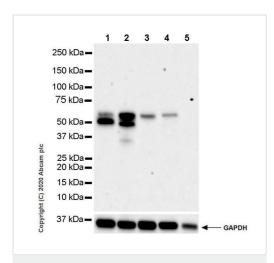
Negative control: Daudi (PMID: 24070417).

<u>ab195889</u> anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor $^{(\!g\!)}$ 594) was used to counterstain tubulin at 1/200 (2.5µg/ml) dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 (2µg/ml) dilution.

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Daudi (Human Burkitt's lymphoma lymphoblast) (Left panel) / T-47D (Human ductal breast epithelial tumor epithelial cell) (Right panel) cells labelling HAI-1 with ab272721 at 1/600 dilution (0.1µg) (Red) compared with a Rabbit monoclonal lgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit lgG (Alexa Fluor[®] 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Negative control: Daudi (PMID: 24070417).



Western blot - Anti-HAl-1 antibody [EPR23294-49] (ab272721)

All lanes : Anti-HAI-1 antibody [EPR23294-49] (ab272721) at 1/1000 dilution

Lane 1: Mouse large intestine tissue lysate

Lane 2: Mouse small intestine tissue lysate

Lane 3: Mouse placenta tissue lysate

Lane 4: Mouse stomach tissue lysate

Lane 5: Mouse liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

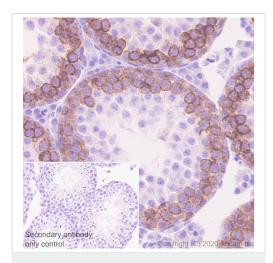
Predicted band size: 58 kDa **Observed band size:** 56,66 kDa

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

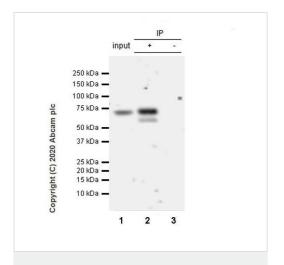
Low expression tissue: liver (PMID:21840293).

The molecular weight observed is consistent with what has been described in the literature (PMID:11013244).

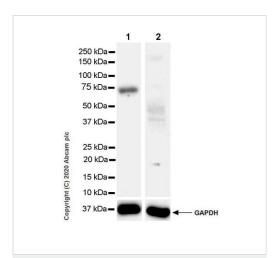
Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-HAI-1 antibody
[EPR23294-49] (ab272721)



Immunoprecipitation - Anti-HAI-1 antibody [EPR23294-49] (ab272721)



Western blot - Anti-HAl-1 antibody [EPR23294-49] (ab272721)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling HAI-1 with ab272721 at 1/2000 followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on germ cells of mouse testis (PMID: 16740973). The section was incubated with ab272721 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 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.

HAI-1 was immunoprecipitated from mouse placenta tissue lysate with ab272721 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab272721 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

Lane 1: Mouse placenta tissue lysate 10µg.

Lane 2: ab272721 IP in mouse placenta tissue lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab272721 in mouse placenta tissue lysate.

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

Exposure time: 3 minutes.

This blot was developed using a higher sensitivity ECL substrate.

All lanes : Anti-HAI-1 antibody [EPR23294-49] (ab272721) at 1/1000 dilution

Lane 1: Rat placenta tissue lysate

Lane 2: Rat liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 58 kDa **Observed band size:** 66 kDa

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

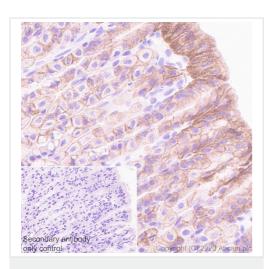
Low expression tissue: liver (PMID:21840293).

Exposure time: 3 minutes.

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling HAI-1 with ab272721 at 1/2000 followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse stomach. The section was incubated with ab272721 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 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 paraffinembedded sections) - Anti-HAI-1 antibody
[EPR23294-49] (ab272721)



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