abcam

Product datasheet

Anti-SIGIRR antibody [EPR22438-93] ab228977

Recombinant RabMAb

3 References 10 Images

Overview

Product name Anti-SIGIRR antibody [EPR22438-93]

Description Rabbit monoclonal [EPR22438-93] to SIGIRR

Host species Rabbit

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

Unsuitable for: ICC/IF

Reacts with: Mouse, Rat, Human Species reactivity

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control IHC-P: Human kidney and colon tissue; Mouse kidney tissue; Rat kidney tissue WB: Hepa1-6 cell

> lysate; Mouse stomach, kidney, spleen and pancreas tissue lysate; Human stomach and fetal kidney lysate; Rat stomach and liver tissue lysate Flow Cyt (intra): MEF and Hepa1-6 cells IP:

Hepa1-6 whole cell lysate

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.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

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

Purity Protein A purified

Clonality Monoclonal

Clone number

EPR22438-93

lgG

Isotype

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab228977 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.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 80 kDa (predicted molecular weight: 46 kDa).
IP		1/30.

Application notes Is unsuitable for ICC/IF.

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Function Acts as a negative regulator of the Toll-like and IL-1R receptor signaling pathways. Attenuates the

recruitment of receptor-proximal signaling components to the TLR4 receptor, probably through an

TIR-TIR domain interaction with TLR4. Through its extracellular domain interferes with the

heterodimerization of II1R1 and IL1RAP.

Tissue specificity Mainly expressed in epithelial tissues such as kidney, lung and gut.

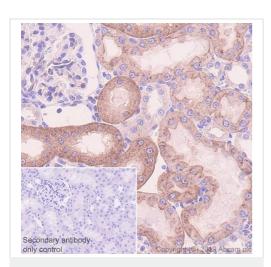
Sequence similarities Belongs to the interleukin-1 receptor family.

Contains 1 lg-like C2-type (immunoglobulin-like) domain.

Contains 1 TIR domain.

Cellular localization Membrane.

Images

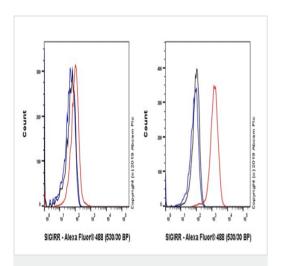


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIGIRR antibody
[EPR22438-93] (ab228977)

Immunohistochemical analysis of paraffin-embedded Rat kidney tissue labeling SIGIRR with ab228977 at 1/1000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat kidney (PMID:14715412, 12925853). The section was incubated with ab228977 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

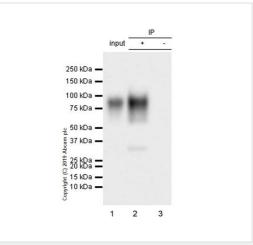


Flow Cytometry (Intracellular) - Anti-SIGIRR antibody [EPR22438-93] (ab228977)

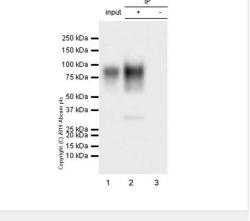
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized MEF (mouse embryonic fibroblast (immortalized)) (Left) / Hepa1-6 (mouse hepatoma epithelial cell line) (Right) cells labelling SIGIRR with ab228977 at 1/600 dilution (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue).

Goat anti rabbit lgG (Alexa Fluor $^{i}\dot{\epsilon}^{1/2}$ 488, <u>ab150077</u>) at 1/2000 dilution was used as the secondary antibody.

Negative control: MEF (PMID 12925853).



Immunoprecipitation - Anti-SIGIRR antibody [EPR22438-93] (ab228977)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIGIRR antibody [EPR22438-93] (ab228977)

IGFBP5 was immunoprecipitated from 0.35 mg Hepa1-6 (mouse hepatoma epithelial cell line) whole cell lysate 10µg with ab254324 at 1/30 dilution. Western blot was performed on the immunoprecipitate using ab228977 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used as the secondary antibody at 1/5000 dilution.

Lane 1: Hepa1-6 (mouse hepatoma epithelial cell) whole cell lysate 10µg

Lane 2: ab254324 IP in Hepa1-6 whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab228977 in Hepa1-6 whole cell lysate.

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

Exposure time: 5 seconds.

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling SIGIRR with ab228977 at 1/1000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse kidney (PMID:14715412, 12925853). The section was incubated with ab228977 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

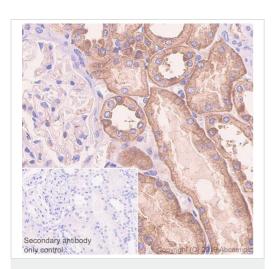


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIGIRR antibody
[EPR22438-93] (ab228977)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling SIGIRR with ab228977 at 1/1000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human colon (PMID:21077278) The section was incubated with ab228977 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SIGIRR antibody
[EPR22438-93] (ab228977)

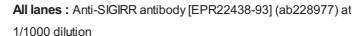
Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling SIGIRR with ab228977 at 1/1000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human kidney (PMID:14715412, 12925853) The section was incubated with ab228977 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

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

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).



Western blot - Anti-SIGIRR antibody [EPR22438-93] (ab228977)



Lane 1: Mouse kidney tissue lysate

Lane 2: Mouse kidney treated lysate treated with with PNGase F

Lysates/proteins at 20 µg per lane.

Secondary

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

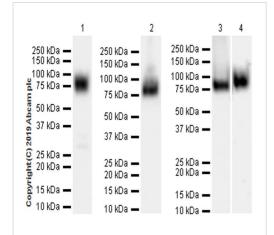
Predicted band size: 46 kDa

Observed band size: 46, 80 kDa

The molecular weight observed is consistent with what has been described in the literature (PMID: 26344057).

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

Exposure time: 92 seconds.



Western blot - Anti-SIGIRR antibody [EPR22438-93] (ab228977)

All lanes : Anti-SIGIRR antibody [EPR22438-93] (ab228977) at 1/1000 dilution

Lane 1: Human fetal kidney tissue lysate

Lane 2: Rat liver tissue lysate

Lane 3: Mouse kidney tissue lysate

Lane 4: Mouse spleen tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

Lane 1 : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution (VeriBlot for IP secondary antibody (HRP))

Lanes 2-4: Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 46 kDa **Observed band size:** 80 kDa

The different apparent molecular weights are due to different levels of glycosylation. (PMID: 26344057).

Exposure times.

Lane 1: 48 seconds; Lane 2: 7.75 seconds; Lane 3: 70 seconds;

Lane 4: 3 minutes.

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

1 2 3 4 5 6

250 kDa —

150 kDa —

100 kDa —

100 kDa —

975 kDa —

975 kDa —

20 kDa —

15 kDa —

10 kDa —

4—GAPDH

Western blot - Anti-SIGIRR antibody [EPR22438-93] (ab228977)

All lanes : Anti-SIGIRR antibody [EPR22438-93] (ab228977) at 1/1000 dilution

Lanes 1 & 5 : Hepa1-6 (mouse hepatoma epithelial cell), whole cell lysate

Lane 2 : MEF (immortalized mouse embryonic fibroblasts), whole cell lysate

Lane 3 : Mouse stomach tissue lysate

Lane 4 : Mouse pancreas tissue lysate

Lane 6: Rat stomach tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

Lanes 1-4 & 6 : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

 $\label{lambda} \textbf{Lane 5: Goat Anti-Rabbit IgG H&L (HRP) ($\underline{ab97051}$) at $1/100000$ dilution (Human stomach tissue lysate)}$

Predicted band size: 46 kDa **Observed band size:** 80 kDa

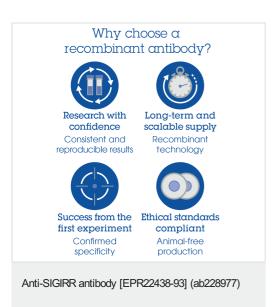
The different apparent molecular weights are due to different levels of glycosylation. (PMID: 26344057).

Negative control: MEF (PMID 12925853)

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

Exposure times.

Lane 1: 15 seconds; Lanes 2-6: 3 minutes.



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