abcam

Product datasheet

Anti-IRS1+IRS2 antibody [EP263Y] ab40777

Recombinant RabMAb

20 References 9 Images

Overview

Product name Anti-IRS1+IRS2 antibody [EP263Y]

Description Rabbit monoclonal [EP263Y] to IRS1 + IRS2

Host species Rabbit

Specificity The immunogen used for this product shares 78% homology with IRS2. Cross-reactivity with this

protein has not been confirmed experimentally.

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

Unsuitable for: IP

Reacts with: Human Species reactivity

Immunogen Synthetic peptide within Human IRS1 (C terminal). The exact sequence is proprietary.

Positive control WB: HeLa and HEK293 cell lysates, HEK-293T transfected with a human IRS1 expression vector

> containing a Myc/DDK tag whole cell lysate, HEK-293T transfected with a human IRS2 expression vector containing a Myc/DDK tag whole cell lysate. IHC-P: Human breast carcinoma and cervical

carcinoma tissues, ICC/IF: MCF-7 cells.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

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.

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

1

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity Protein A purified

ClonalityMonoclonalClone numberEP263Y

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab40777 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

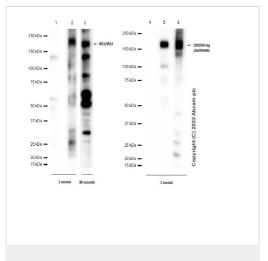
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Detects a band of approximately 170 kDa (predicted molecular weight: 130 kDa).
ICC/IF		1/250 - 1/500.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.

Application notes Is unsuitable for IP.

Target

Cellular localization IRS2: Cytoplasm > cytosol.

Images



Western blot - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

Lanes 1-3: Anti-IRS1+IRS2 antibody [EP263Y] (ab40777) at 1/1000 dilution

Lanes 4-6 : Anti-DDDDK tag (Binds to FLAG® tag sequence) antibody [EPR20018-251] (ab205606) at 1/1000 dilution

Lanes 1 & 4: HEK-293T transfected with an empty vector whole cell lysate

Lanes 2 & 5: HEK-293T transfected with a human IRS1 expression vector containing a Myc/DDK tag whole cell lysate

Lanes 3 & 6: HEK-293T transfected with a human IRS2 expression vector containing a Myc/DDK tag whole cell lysate

Lysates/proteins at 20 µg per lane.

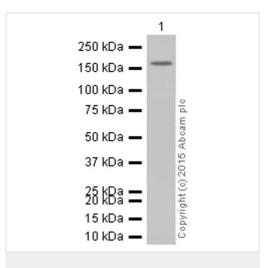
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 130 kDa **Observed band size:** 170 kDa

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

Exposure time: Lane 1, 2, 4, 5, 6: 1 second. Lane 3: 80 seconds.



Western blot - Anti-IRS1 antibody [EP263Y] (ab40777)

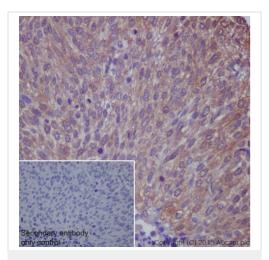
Anti-IRS1+IRS2 antibody [EP263Y] (ab40777) at 1/10000 dilution (purified) + HEK293 whole cell lysate at 10 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

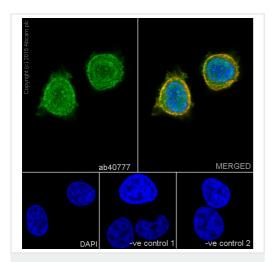
Predicted band size: 130 kDa **Observed band size:** 170 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRS1 antibody [EP263Y] (ab40777)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling IRS1 with purified ab40777 at a dilution of 1/100. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

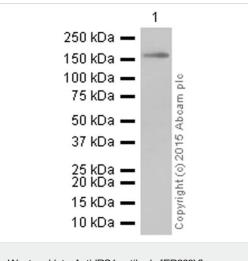


Immunocytochemistry/ Immunofluorescence - Anti-IRS1 antibody [EP263Y] (ab40777)

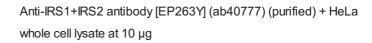
Immunocytochemistry/Immunofluorescence analysis of MCF-7 cells labelling IRS1 with purified ab40777 at a dilution of 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat antirabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse antitubulin (1/1000) and ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/250) and secondary antibody, **ab150120**, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: <u>ab7291</u> (1/1000) and secondary antibody, <u>ab150077</u>, an Alexa Fluor $^{\otimes}$ 488-conjugated goat anti-rabbit lgG (1/1000).



Western blot - Anti-IRS1 antibody [EP263Y] (ab40777)

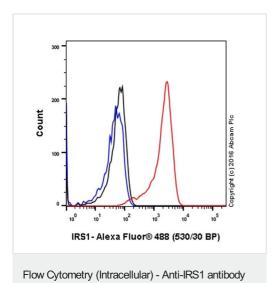


Secondary

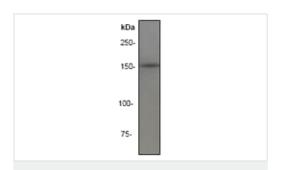
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 130 kDa **Observed band size:** 170 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Intracellular Flow Cytometry analysis of MCF-7 (human breast carcinoma) cells labeling IRS1 with unpurified ab40777 at 1/90 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr[®] 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.

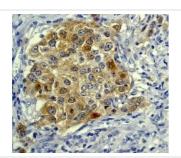


[EP263Y] (ab40777)

Western blot - Anti-IRS1 antibody [EP263Y] (ab40777)

Anti-IRS1+IRS2 antibody [EP263Y] (ab40777) at 1/1000 dilution (unpurified) + HeLa cell lysate at 10 µg

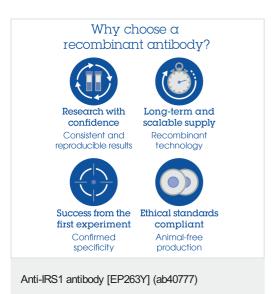
Predicted band size: 130 kDa **Observed band size:** 170 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRS1 antibody [EP263Y] (ab40777)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma labelling IRS1 with unpurified ab40777.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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