abcam

ab40777

Anti-IRS1+IRS2 antibody [EP263Y]

Recombinant | RabMAb | 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	EP263Y
Purification technique	Affinity purification Protein A
Specificity	The immunogen used for this product shares 78% homology with IRS2. Cross-reactivity with this protein has not been confirmed experimentally.
Concentration	0.991 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/50 - 1/100

Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

IP

Not recommended

Species	Human, Recombinant fragment - Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Species	Recombinant fragment - Human
Dilution info	1/1000 - 1/10000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250 - 1/500
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Recombinant fragment - Human
Dilution info	-
Notes	-

Target data

See full target information Insulin receptor substrate 1

Function

Signaling adapter protein that participates in the signal transduction from two prominent receptor tyrosine kinases, insulin receptor/INSR and insulin-like growth factor I receptor/IGF1R (PubMed:7541045, PubMed:33991522, PubMed:38625937). Plays therefore an important role in development, growth, glucose homeostasis as well as lipid metabolism (PubMed:19639489). Upon phosphorylation by the insulin receptor, functions as a signaling scaffold that propagates insulin action through binding to SH2 domain-containing proteins including the p85 regulatory subunit of PI3K, NCK1, NCK2, GRB2 or SHP2 (PubMed:11171109, PubMed:8265614). Recruitment of GRB2 leads to the activation of the guanine nucleotide exchange factor SOS1 which in turn triggers the Ras/Raf/MEK/MAPK signaling cascade (By similarity). Activation of the PI3K/AKT pathway is responsible for most of insulin metabolic effects in the cell, and the Ras/Raf/MEK/MAPK is involved in the regulation of gene expression and in cooperation with the PI3K pathway regulates cell growth and differentiation. Acts a positive regulator of the Wnt/beta-catenin signaling pathway through suppression of DVL2 autophagy-mediated degradation leading to cell proliferation (PubMed:24616100).

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

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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	The targets IRS1 and IRS2 also known as Insulin Receptor Substrate 1 and 2 are important adaptor proteins that play an important role in insulin signaling. IRS1 and IRS2 have molecular masses of approximately 180 kDa and 185 kDa respectively. These proteins are expressed in a wide range of tissues but show particularly high expression in insulin-responsive tissues such as muscle liver and adipose tissues. IRS1 and IRS2 function by interacting with activated insulin receptors allowing subsequent recruitment of downstream signaling molecules.
Biological function summary	IRS1 and IRS2 translate insulin receptor activation into metabolic and mitogenic effects. These proteins form signaling complexes that mediate the action of insulin

	and insulin-like growth factor 1 (IGF-1). IRS1 and IRS2 interact with several signaling molecules including PI3K and GRB2 which are essential for the activation of downstream pathways. Their role is essential in glucose homeostasis cell growth and differentiation. In some contexts IRS1 and IRS2 also participate in signaling pathways influenced by other growth factors.
Pathways	IRS1 and IRS2 have pivotal roles in the insulin signaling and IGF-1 signaling pathways. They facilitate signal transduction from the insulin and IGF-1 receptors impacting both metabolic and growth-related pathways. Within these pathways their interaction with PI3K highlights their importance in glucose uptake and utilization while their connection with SHC and ERK pathways emphasizes their involvement in cell growth and proliferation. The coordinated action of IRS1 and IRS2 helps maintain energy balance and cellular functions.
Associated diseases and disorders	IRS1 and IRS2 have significant associations with type 2 diabetes and certain cancers. In type 2 diabetes alterations in IRS1 and IRS2 signaling can impair glucose metabolism leading to insulin resistance. Genetic variations or dysregulation in their function often contribute to diabetes susceptibility. Additionally IRS1 and IRS2 are linked to cancer as their pathways overlap with pathways that influence cell growth and survival. Increased signaling can enhance cell proliferation and resistance to apoptosis with relevant proteins like PI3K and AKT further involved in these processes.

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

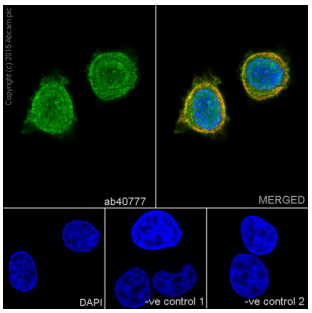
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images

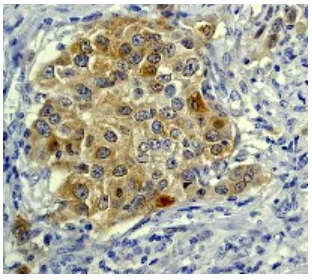


Immunocytochemistry/ Immunofluorescence - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

IRS1+IRS2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-IRS1+IRS2 antibody

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 anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291 a mouse anti-tubulin (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).

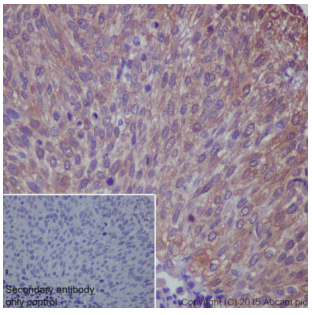
Control 2: ab7291 (1/1000) and secondary antibody ab150077 an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

IRS1+IRS2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IRS1+IRS2 antibody

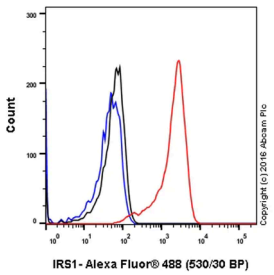
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma labelling IRS1 with unpurified ab40777.
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

IRS1+IRS2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-IRS1+IRS2 antibody

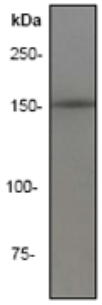
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling IRS1 with purified ab40777 at a dilution of 1/100. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051 a HRP-conjugated goat anti-rabbit IgG (H+L) was used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



Flow Cytometry (Intracellular) - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

IRS1+IRS2 Flow Cytometry (Intracellular) staining using rabbit Anti-IRS1+IRS2 antibody

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.



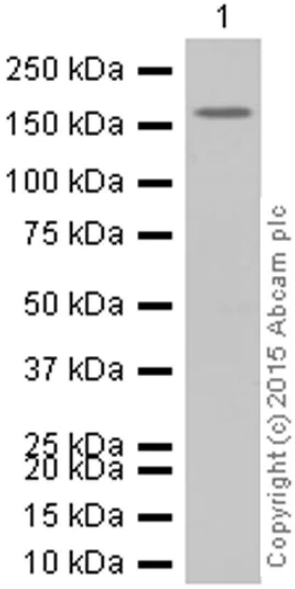
Western blot - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

IRS1+IRS2 Western blot staining of HeLa cell lysate using rabbit Anti-IRS1+IRS2 antibody

All lanes:
Western blot - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777) at 1/1000 dilution

All lanes:
HeLa cell lysate at 10 µg

Observed band size: 170 kDa



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

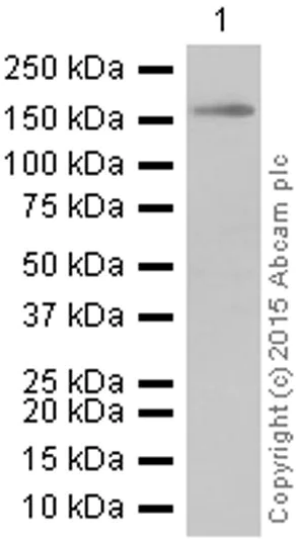
Blocking and dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777) at 1/10000 dilution

All lanes:
HEK293 whole cell lysate at 10 µg

Secondary

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

Observed band size: 170 kDa



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

Blocking and dilution buffer: 5% NFDM/TBST
All lanes:
Western blot - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777)

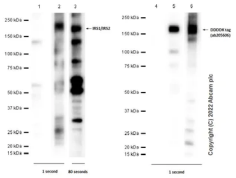
All lanes:
HeLa whole cell lysate at 10 µg

Secondary

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

Predicted band size: 52 kDa

Observed band size: 170 kDa



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

Blocking and diluting buffer and concentration: 5% NFDM /TBST. Exposure time: Lane 1, 2, 4, 5 , 6: 1 second. Lane 3: 80 seconds.
Lanes 1 - 3:
Western blot - Anti-IRS1+IRS2 antibody [EP263Y] (ab40777) at 1/1000 dilution

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

Lanes 1 and 4:
HEK-293T transfected with an empty vector whole cell lysate at 20 µg

Lanes 2 and 5:
HEK-293T transfected with a human IRS1 expression vector containing a Myc/DDK tag whole cell lysate at 20 µg

Lanes 3 and 6:
HEK-293T transfected with a human IRS2 expression vector containing a Myc/DDK tag 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: 170 kDa

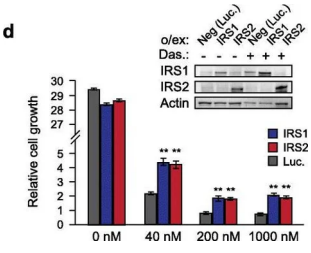


Image collected and cropped by CiteAb under a

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

IRS1+IRS2 Western blot staining using rabbit Anti-IRS1+IRS2 antibody

Western Blotting using Anti-IRS1+IRS2 antibody [EP263Y], ab40777. Publication image from Eyler, C. E. et al., 2020, Genome Biol, 32669109. Legend direct from paper.

Analysis of clonal cultures identifies distinct but functionally parallel chromosomal amplifications in “jackpot” drug resistant lineages. a Schematic depicts isolation of single cells and derivation of clonal cultures from multiple dasatinib-treated replicates of Experiments #1 (day 70; left) and 2

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(day 56; right). b Chart details characteristics of those clonal cultures isolated from dasatinib experiments that were analyzed by low-coverage whole-genome sequencing (WGS), compared to drug-naïve GSCs (top row). Clones that exhibited features consistent with induced drug persistence (epigenetic) in these experiments are shown in the top part of chart. Clones that exhibited features consistent with genetic (acquired or preexisting) resistance are shown in the bottom part of chart. For each single-cell-derived clone, the chart indicates the experiment and replicate from which it was derived (Clone ID), the DNA barcode identity (Lineage ID), and the rank and abundance of the respective lineage in the gDNA analysis of the corresponding experiment (day 70 for Experiment #1; day 56 for Experiment #2). Genomic tracks depict read density in low-coverage WGS over chromosomal bands 13q34 (containing IRS2) and 2q36 (containing IRS1). The final column summarizes the extent to which the clone loses dasatinib resistance after a drug-free washout period. c Single-cell-derived clones were subjected to a drug-free washout period and then re-challenged with dasatinib. Plot depicts growth in the presence of dasatinib, relative to drug-naïve GSC8 cells (Y-axis); all conditions were treated independently in at least 4 replicates. Circles represent the mean relative growth of individual clonal cultures. Horizontal black bars reflect the mean growth of clones from the indicated lineage (top). d Barplots depict relative growth of drug-naïve GSC8 cells transduced with IRS1, IRS2, or luciferase expression constructs (**, $p < 0.01$ by two-tailed Student’ s t test; standard error bars depicted). Cells were grown at the indicated dasatinib concentrations. Western blot shows IRS1, IRS2, and Actin protein expression in the indicated GSC8 cultures. Overexpression of IRS1 or IRS2 confers dasatinib resistance

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