abcam

Product datasheet

Anti-IRS2 antibody [EPR904(2)] ab134101

敲除验证 重组 RabMAb

26 References 13 图像

概述

产品名称	Anti-IRS2抗体[EPR904(2)]
描述	兔单克隆抗体[EPR904(2)] to IRS2
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide within Human IRS2 aa 1300 to the C-terminus (C terminal). The exact sequence is proprietary.
阳性对照	WB: HEK293, HEK293 (treated with insulin), and NIH/3T3 (treated with insulin) and SH-SY5Y cell lysates; A375 (human malignant melanoma epithelial cell) and A549 whole cell lysates IHC-P: Human kidney, breast and muscle tissues and rat kidney tissue. ICC/IF: SH-SY5Y cells. HEK293 WT cells (HEK293-IRS2 KO used as a negative cell line). Flow Cyt (intra): HeLa cells.
常规说明	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**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR904(2)
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab134101于以下的经测试应用**

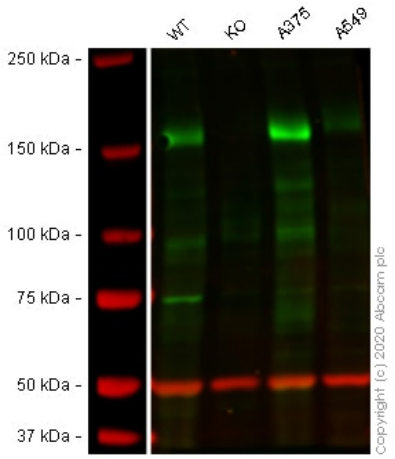
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/100 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/1000 - 1/10000. Detects a band of approximately 170-185 kDa (predicted molecular weight: 137 kDa). Although some papers support the expression in liver (PMID: 30202052), A549 (PMID: 30988063), NCI-H1299 (PMID: 30988063), LADMAC (PMID: 29115630), MDA-MB-231 (PMID: 29685905) and MEF (PMID: 30679431), ab134101 can't detect the target band in these samples, even at the dilution of 1:200
IHC-P		1/50 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/300.

靶标

功能	May mediate the control of various cellular processes by insulin.
序列相似性	Contains 1 IRS-type PTB domain. Contains 1 PH domain.
翻译后修饰	Phosphorylated upon DNA damage, probably by ATM or ATR.
细胞定位	Cytoplasm > cytosol.

图片



Western blot - Anti-IRS2 antibody [EPR904(2)] (ab134101)

All lanes : Anti-IRS2 antibody [EPR904(2)] (ab134101) at 1/1000 dilution

Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 2 : IRS2 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : A-375 cell lysate
Lane 4 : A549 (Human lung carcinoma cell line) whole cell lysate

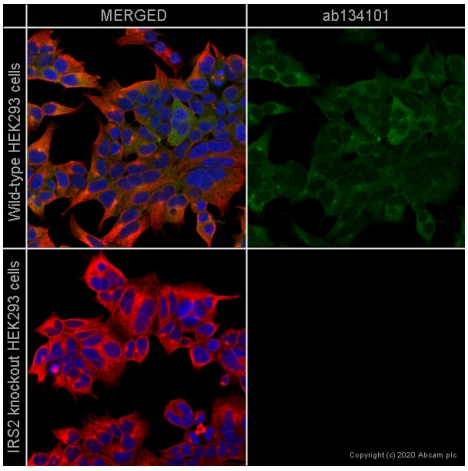
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 137 kDa
Observed band size: 160 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab134101 observed at 160 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

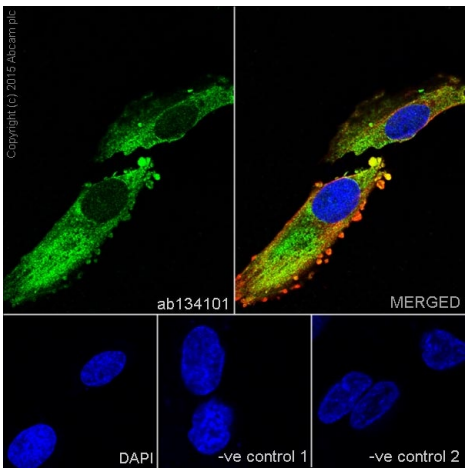
ab134101 was shown to react with IRS2 in wild-type HEK-293 cells in western blot with loss of signal observed in IRS2 knockout sample. Wild-type and IRS2 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab134101 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-IRS2 antibody [EPR904(2)] (ab134101)

ab134101 staining IRS2 in wild-type HEK293 cells (top panel) and IRS2 knockout HEK293 cells (bottom panel). The cells were fixed with 4% paraformaldehyde (10 min) permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab134101 at 1/500 and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

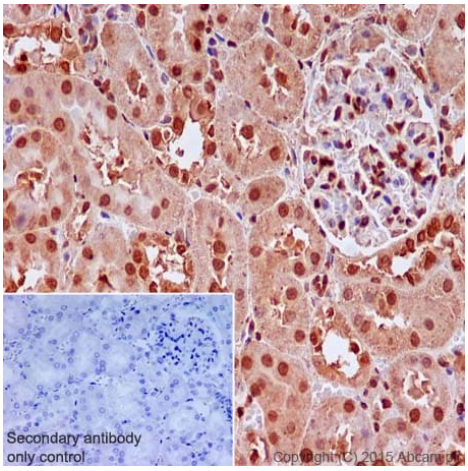


Immunocytochemistry/ Immunofluorescence - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Immunocytochemistry/Immunofluorescence analysis of SH-SY5Y cells labelling IRS2 with purified ab134101 at 1/300. 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.

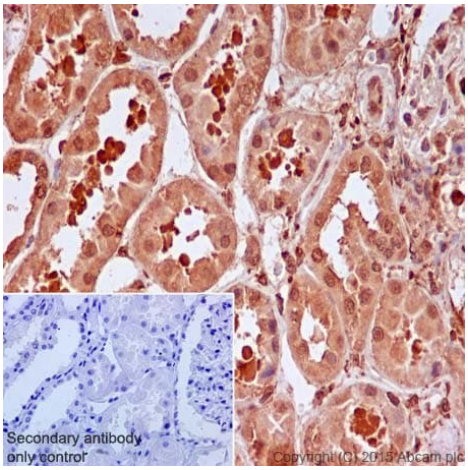
Control 1: primary antibody (1/300) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

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



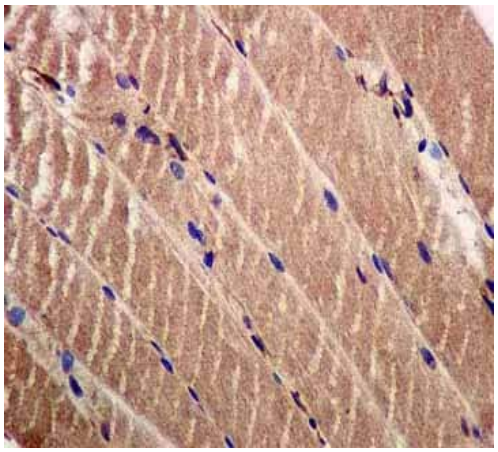
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue labelling IRS2 with purified ab134101 at 1/500. Heat mediated antigen retrieval was performed using Tris/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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRS2 antibody [EPR904(2)] (ab134101)

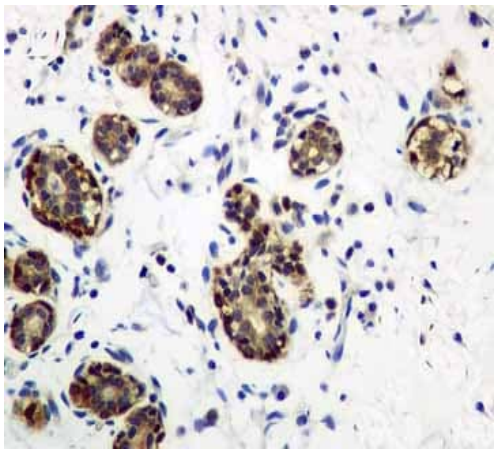
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling IRS2 with purified ab134101 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human muscle tissue labelling IRS2 with unpurified ab134101 at a dilution of 1/50.

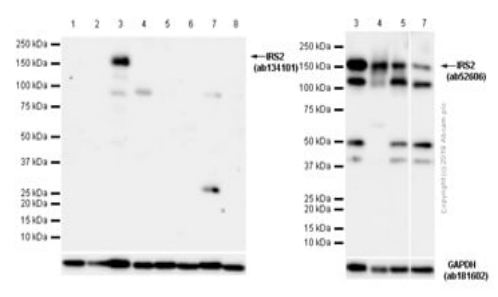
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast tissue labelling IRS2 with unpurified ab134101 at a dilution of 1/50.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-IRS2 antibody [EPR904(2)] (ab134101)

All lanes : Anti-IRS2 antibody [EPR904(2)] (ab134101) at 1/1000 dilution

Lane 1 : Rat liver lysates
Lane 2 : Human liver lysates
Lane 3 : A375 (human malignant melanoma epithelial cell) whole cell lysates
Lane 4 : A549 (human lung carcinoma epithelial cell) whole cell lysates
Lane 5 : NCI-H1299 (human lung carcinoma epithelial cell) whole cell lysates
Lane 6 : LADMAC (Mouse bone marrow monocyte macrophage) whole cell lysates

Lane 7 : MDA-MB-231 (human breast adenocarcinoma epithelial cell) whole cell lysates
Lane 8 : MEF (Mouse embryonic fibroblast) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Developed using the ECL technique.

Predicted band size: 137 kDa
Observed band size: 170-185 kDa

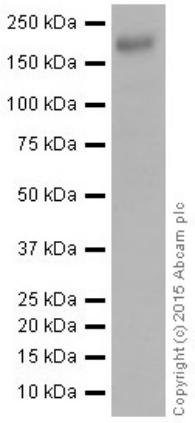
Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

Exposure time:

Left image: 180 seconds

Right image: 40 seconds

Although some papers support the expression in liver (PMID: 30202052), A549 (PMID: 30988063), NCI-H1299 (PMID: 30988063), LADMAC (PMID: 29115630), MDA-MB-231 (PMID: 29685905) and MEF (PMID: 30679431), ab134101 can't detect the target band in these samples, even at the dilution of 1:200.



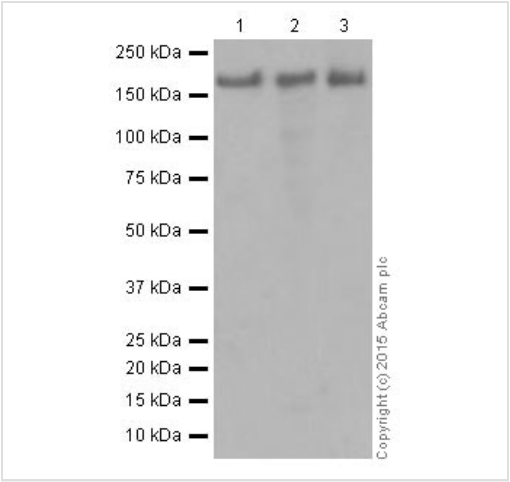
Western blot - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Anti-IRS2 antibody [EPR904(2)] (ab134101) at 1/5000 dilution (purified) + NIH/3T3 whole cell lysate - treated with insulin at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 137 kDa
Observed band size: 170-185 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-IRS2 antibody [EPR904(2)] (ab134101)

All lanes : Anti-IRS2 antibody [EPR904(2)] (ab134101) at 20 µg (purified)

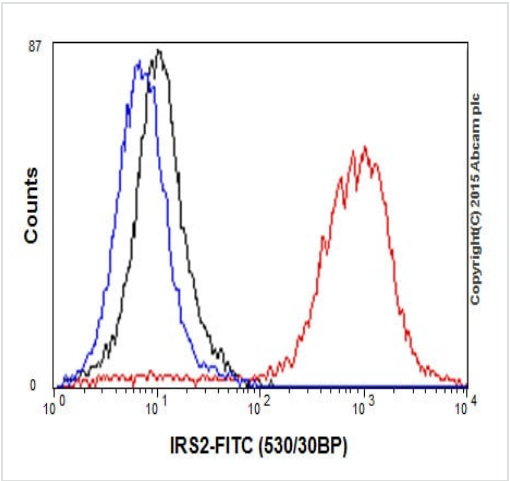
Lane 1 : HEK293 whole cell lysate - untreated
Lane 2 : HEK293 whole cell lysate - treated with insulin
Lane 3 : SH-SY5Y whole cell lysate

Lysates/proteins at 20 µg per lane.

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

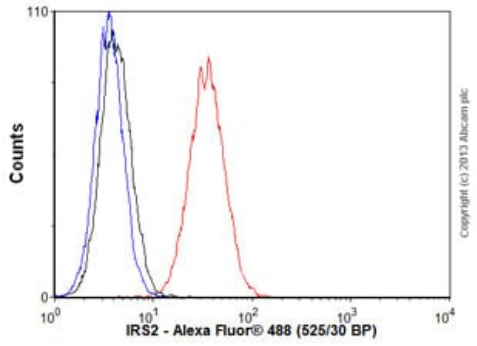
Predicted band size: 137 kDa
Observed band size: 170-185 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Intracellular Flow Cytometry analysis of HeLa cells labelling IRS2 with purified ab134101 at 1/120 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Flow Cytometry (Intracellular) - Anti-IRS2 antibody [EPR904(2)] (ab134101)

Overlay histogram showing HeLa cells stained with unpurified ab134101 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab134101, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Anti-IRS2 antibody [EPR904(2)] (ab134101)

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