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Anti-IRS1 Antibody Picoband™ (monoclonal, 10I3)

Catalog # ABO14872

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P35568
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-IRS1 Antibody Picoband™ (monoclonal, 10I3) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	3667
Other Names	Insulin receptor substrate 1, IRS-1, IRS1
Calculated MW	131591
Application Details	Western blot, 0.1-0.5 □g/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 □g/ml Immunocytochemistry/Immunofluorescence, 2 □g/ml Flow Cytometry, 1-3 □g/1x10^6 cells
Subcellular Localization	Cytosol. Nucleus. Caveola. Insulin receptor complex. Plasma membrane.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na_2HPO_4, 0.05mg NaN_3.
Clone Names	Clone: 10I3
Immunogen	E.coli-derived human IRS1 recombinant protein (Position: S1041-Q1242). Human IRS1 shares 78% and 80% amino acid (aa) sequence identity with mouse and rat IRS1, respectively.
Cross Reactivity	No cross-reactivity with other proteins.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	IRS1
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).
Cellular Location	Cytoplasm. Nucleus. Note=Nuclear or cytoplasmic localization of IRS1 correlates with the transition from proliferation to chondrogenic differentiation.

Background

Insulin receptor substrate 1 (IRS-1) is a signalling adapter protein that in humans is encoded by the IRS-1 gene. It is mapped to 2q36.3. This gene exhibited no intrinsic enzyme activity, and it can serve as a docking protein involved in binding and activating other signal transduction molecules after being phosphorylated on tyrosine by insulin receptor kinase. IRS1 plays a key role in transmitting signals from the insulin and insulin-like growth factor-1 (IGF-1) receptors to intracellular pathways PI3K/Akt and Erk MAP kinase pathways. IRS1 also has important biological function for both metabolic and mitogenic (growth promoting) pathways. In addition to those, IRS1 is a key regulator of PI3K within malignant cells.

Images

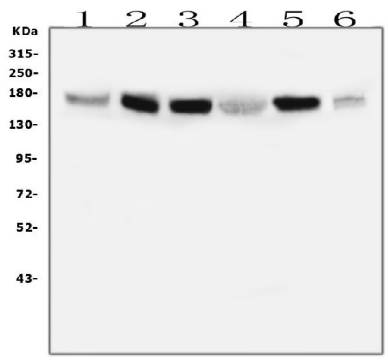


Figure 1. Western blot analysis of IRS1 using anti-IRS1 antibody (M00268-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human A549 whole cell lysates
Lane 2: human T-47D whole cell lysates
Lane 3: human Caco-2 whole cell lysates
Lane 4: human SW620 whole cell lysates
Lane 5: human Hela whole cell lysates
Lane 6: human Raji whole cell lysates
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-IRS1 antigen affinity purified monoclonal antibody (Catalog # M00268-1) at 0.5 µg/mL overnight at 4°C, then

washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for IRS1 at approximately 160-180KD. The expected band size for IRS1 is at 130KD.

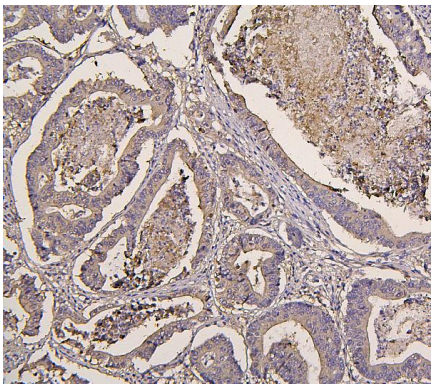


Figure 2. IHC analysis of IRS1 using anti-IRS1 antibody (M00268-1).
IRS1 was detected in paraffin-embedded section of human colon cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-IRS1 Antibody (M00268-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

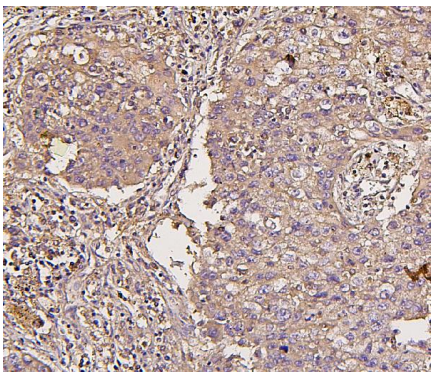


Figure 3. IHC analysis of IRS1 using anti-IRS1 antibody (M00268-1).
IRS1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-IRS1 Antibody (M00268-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

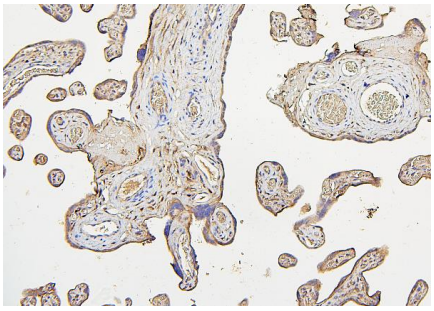
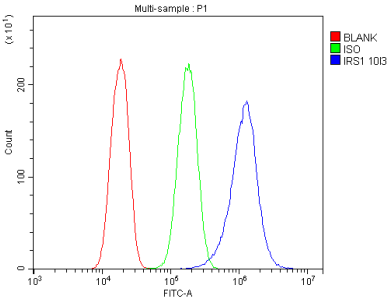


Figure 4. IHC analysis of IRS1 using anti-IRS1 antibody (M00268-1).
IRS1 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-IRS1 Antibody (M00268-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

Figure 5. Flow Cytometry analysis of PC-3 cells using anti-IRS1 antibody (M00268-1).
Overlay histogram showing PC-3 cells stained with M00268-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRS1 Antibody (M00268-1,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary



antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

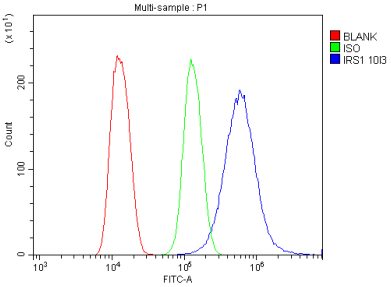


Figure 6. Flow Cytometry analysis of U20S cells using anti-IRS1 antibody (M00268-1).
Overlay histogram showing U20S cells stained with M00268-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRS1 Antibody (M00268-1,1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

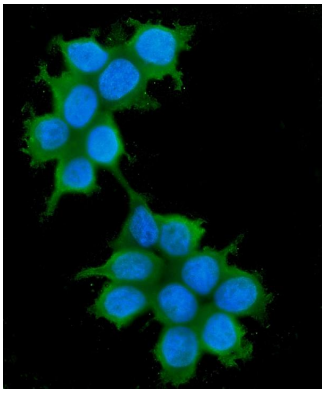


Figure 7. IF analysis of IRS1 using anti-IRS1 antibody (M00268-1).
IRS1 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 µg/mL mouse anti-IRS1 Antibody (M00268-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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