

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

Catalog Number: M00268-1

About IRS1

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.

Overview

Product Name	Anti-IRS1 Antibody Picoband™ (monoclonal, 10I3)
Reactive Species	Human
Description	Boster Bio Anti-IRS1 Antibody Picoband™ (monoclonal, 10I3) catalog # M00268-1. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 10I3
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Storage Instructions	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.
Host	Mouse
Uniprot ID	P35568

Technical Details

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.
Predicted Reactive Species	Hepatitis Virus
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2a
Form	Lyophilized
Concentration	0

Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml</p> <p>Immunocytochemistry/Immunofluorescence, 2ug/ml</p> <p>Flow Cytometry, 1-3ug/1x10⁶ cells</p>

Anti-IRS1 Antibody Picoband™ (monoclonal, 10I3) (M00268-1) Images

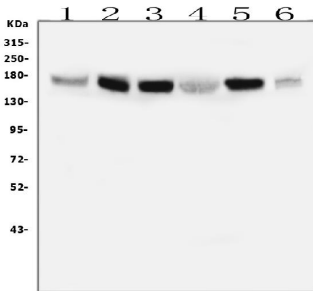


Figure 1. Western blot analysis of IRS1 using anti-IRS1 antibody (M00268-1).

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 ug/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.

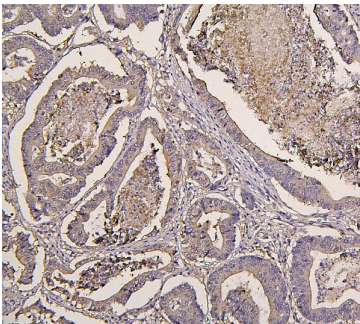


Figure 2. IHC analysis of IRS1 using anti-IRS1 antibody (M00268-1).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

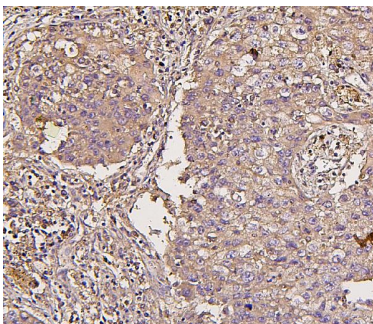


Figure 3. IHC analysis of IRS1 using anti-IRS1 antibody (M00268-1).

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 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

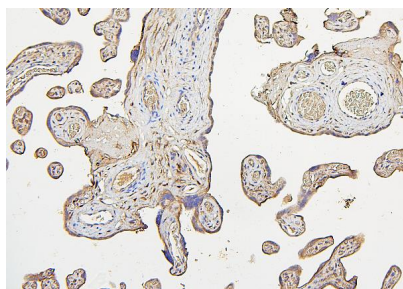


Figure 4. IHC analysis of IRS1 using anti-IRS1 antibody (M00268-1).
IRS1 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/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 Streptavidin-Biotin-Complex (SABC)(Catalog # SA1021) with DAB as the chromogen.

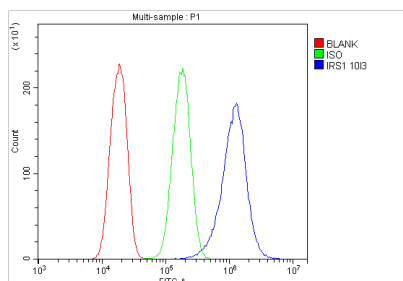


Figure 5. Flow Cytometry analysis of PC-3 cells using anti-IRS1 antibody (M00268-1).
Overlay histogram showing PC-3 cells stained with M00268-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRS1 Antibody (M00268-1,1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

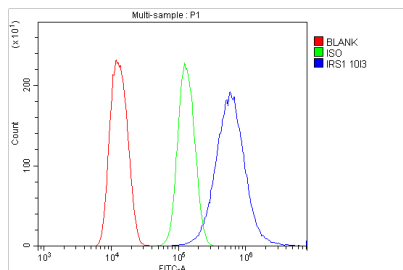


Figure 6. Flow Cytometry analysis of U2OS cells using anti-IRS1 antibody (M00268-1).
Overlay histogram showing U2OS cells stained with M00268-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRS1 Antibody (M00268-1,1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

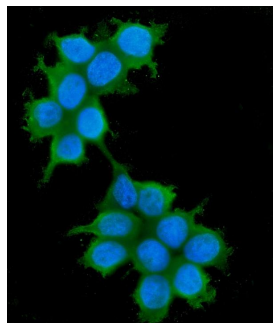


Figure 7. IF analysis of IRS1 using anti-IRS1 antibody (M00268-1).
IRS1 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/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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