

## Product Datasheet

### Anti-IRS1 Antibody (monoclonal, 10I3) (orb570313)

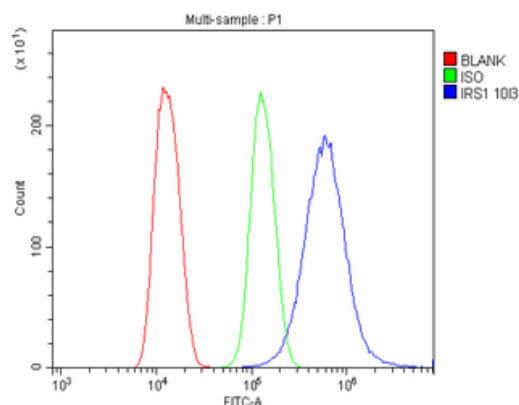
<b>Description</b>	Anti-IRS1 Antibody (monoclonal, 10I3). Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
<b>Species/Host</b>	Mouse
<b>Reactivity</b>	Human
<b>Conjugation</b>	Unconjugated
<b>Tested Applications</b>	FC, ICC, IF, IHC, WB
<b>Immunogen</b>	E.coli-derived human IRS1 recombinant protein (Position: S1041-Q1242). Human IRS1 shares 78% and 80% amino acid (aa) sequence identity with mouse and rat IRS1, respectively.
<b>Form/Appearance</b>	Lyophilized
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Application notes</b>	Western blot, 0.1-0.5µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1µg/ml Immunocytochemistry/Immunofluorescence, 2µg/ml Flow Cytometry (Fixed), 1-3µg/1x10 <sup>6</sup> cells. Add 0.2ml of distilled water will yield a concentration of 500µg/ml
<b>Isotype</b>	Mouse IgG2a
<b>Clonality</b>	Monoclonal
<b>Clone Number</b>	10I3
<b>Antibody Type</b>	Primary Antibody

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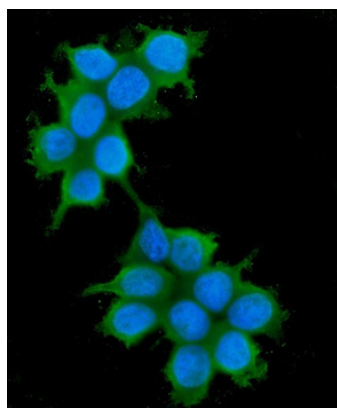
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**MW** 160-180 kDa**Uniprot ID** **P35568****Expiration Date** 12 months from date of receipt.

Flow Cytometry analysis of U2OS cells using anti-IRS1 antibody. Overlay histogram showing U2OS cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRS1 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



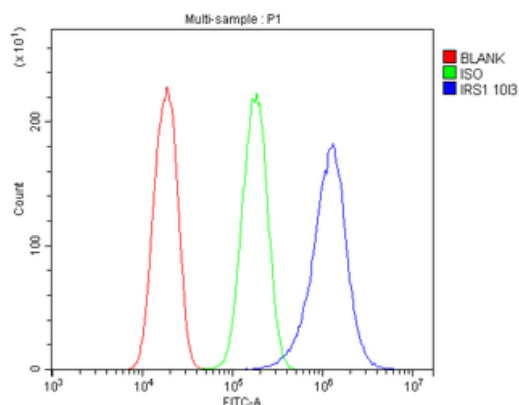
IF analysis of IRS1 using anti-IRS1 antibody. IRS1 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2  $\mu\text{g}/\text{mL}$  mouse anti-IRS1 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG 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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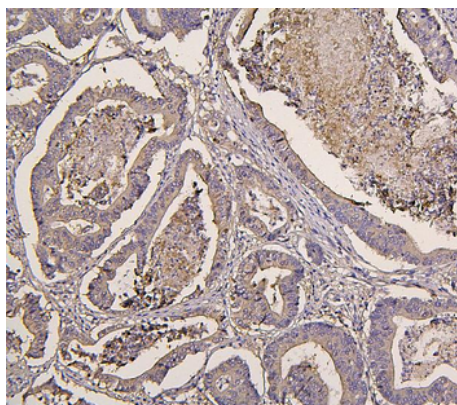
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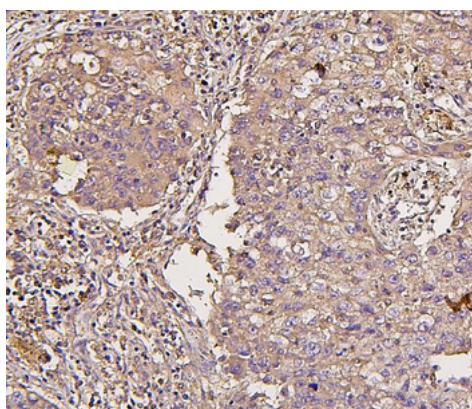
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Flow Cytometry analysis of PC-3 cells using anti-IRS1 antibody. Overlay histogram showing PC-3 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-IRS1 Antibody (1  $\mu\text{g}/1 \times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (5-10  $\mu\text{g}/1 \times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1  $\mu\text{g}/1 \times 10^6$ ) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IHC analysis of IRS1 using anti-IRS1 antibody. 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  $\mu\text{g}/\text{ml}$  mouse anti-IRS1 Antibody 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) with DAB as the chromogen.



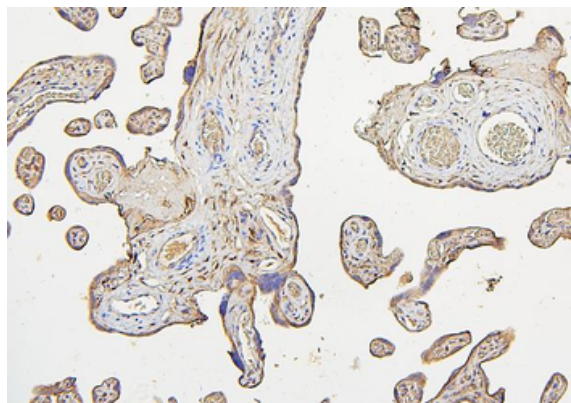
IHC analysis of IRS1 using anti-IRS1 antibody. IRS1 was detected in paraffin-embedded section of human lung cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1  $\mu\text{g}/\text{ml}$  mouse anti-IRS1 Antibody 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) with DAB as the chromogen.

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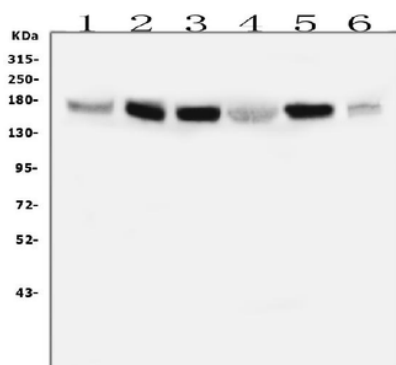
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IHC analysis of IRS1 using anti-IRS1 antibody. IRS1 was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-IRS1 Antibody 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) with DAB as the chromogen.



Western blot analysis of IRS1 using anti-IRS1 antibody. 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 50 ug 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 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 with Tanon 5200 system. A specific band was detected for IRS1 at approximately 160-180KD. The expected band size for IRS1 is at 130KD.

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