

Anti-IRS1 Antibody [SD2046]

ET1612-45



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	132 kDa
Clone number:	SD2046

Description: The insulin receptor substrate-1 (IRS-1), a protein major substrate of the insulin receptor, is phosphorylated in response to stimulation of cells by insulin, insulin-like growth factor 1 (IGF-1) and interleukin 4 (IL-4). IRS-1 is phosphorylated on serine, threonine and tyrosine residues in a variety of tissues. An insulin-sensitive serine/threonine kinase casein kinase II mediates a portion of the insulin-stimulated serine/threonine phosphorylation of overexpressed IRS-1 in vivo. Thr 502 is identified as the major casein kinase II-catalyzed phosphorylation site in rat IRS-1, and Ser 99 is an additional phosphorylation site catalyzed by casein kinase II. Thus, casein kinase II-catalyzed phosphorylation of IRS-1 may be a component of the intracellular insulin signaling cascade. IRS-1 contains three putative binding sites for 14-3-3 (Ser 270, Ser 374 and Ser 641) and the motif around Ser 270 is located in the phosphotyrosine binding domain of IRS-1, which is responsible for the interaction with the insulin receptor. The association of 14-3-3 with IRS-1 increases significantly upon treatment with okadaic acid, a potent serine/ threonine phosphatase inhibitor. Therefore, the association of 14-3-3 protein may play a role in the regulation of insulin sensitivity by interrupting the association between the insulin receptor and IRS-1.

Immunogen:	Synthetic peptide within human IRS1 aa 1160-1242.
Positive control:	SW480, HeLa, MCF-7, human breast carcinoma tissue.
Subcellular location:	Cytoplasm, Nucleus.
Database links:	SwissProt: P35568 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Images

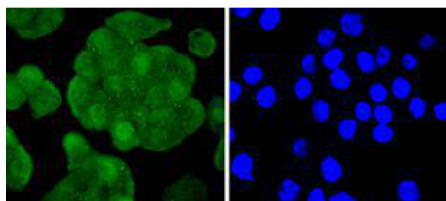


Fig1: ICC staining of IRS1 in SW480 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-45, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

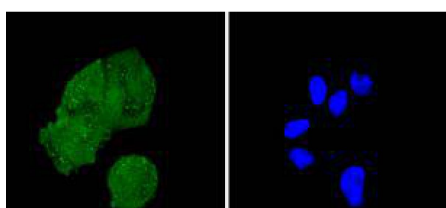


Fig2: ICC staining of IRS1 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-45, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

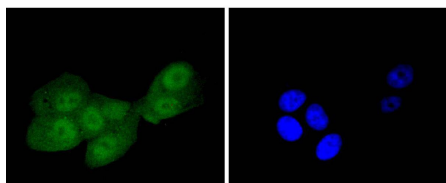


Fig3: ICC staining of IRS1 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1612-45, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

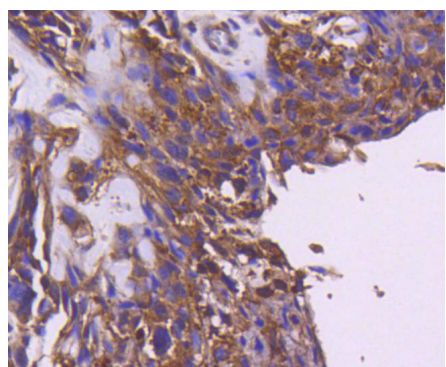


Fig4: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-IRS1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1612-45, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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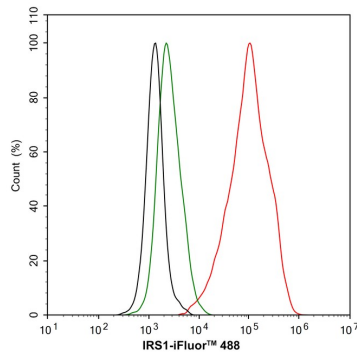


Fig5: Flow cytometric analysis of HeLa cells labeling IRS1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1612-45, 1 μ g/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4 $^{\circ}$ C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4 $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are “FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE”.

Background References

1. Lovly CM et al. Rationale for co-targeting IGF-1R and ALK in ALK fusion-positive lung cancer. *Nat Med* 20:1027-34 (2014).
2. Pan T et al. Cytohesins/ARNO: the function in colorectal cancer cells. *PLoS One* 9:e90997 (2014).

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