

IRS1 [pY612] Rabbit Polyclonal Antibody

Store at 2°C to 8°C (short-term), or -20°C (long-term)

Catalog Number: 44-816G

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Clonality: Polyclonal	Quantity: 10 mini-blot size	Volume : 100 μL
Host/Class: Rabbit IgG	Reactivity: Human IRS1 [pY612]	Predicted Reactivity: Human, Rat, Mouse, Chicken

Product description

Insulin Receptor Substrate 1 (IRS1) is a multi-phosphorylated cytoplasmic docking protein involved in the positive regulation of metabolism and proliferation by insulin, IL-4 and other cytokines. The catalytic domain of the insulin receptor is activated upon binding extracellular binding of ligand, resulting in cytoplasmic binding of IRS proteins. IRS is phosphorylated on tyrosine residues, to recruit a variety of Src homology-2 (SH2) domain-containing proteins and activate PI3-kinase, and MAP kinase signaling pathways. When phosphorylated, tyrosine 612 is a pYMxM consensus motif PI3-kinase binding site, which leads to the activation of the PI3K/AKT signaling pathway.

Product specifications

Immunogen:	A chemically synthesized phosphopeptide derived from the region of human IRS1 containing tyrosine 612	
Purification:	Antibody negatively preadsorbed using a non- phosphopeptide then purified by epitope-specific affinity chromatography	
Apparent MW:	165 kDa	
Sequence Identity:	Human	
Sequence Homology:	Rat, Mouse, Chicken	
Lot:	See product label	

Product applications

The antibody has been used in western blotting (1:1000 dilution). Other applications may work but have not been tested.

Because conditions may vary, it is recommended that each investigator determine the optimal amount of antibody to be used for each application.

Storage and handling

Store the antibody at 2° C to 8° C for up to 1 week, or apportion into working aliquots and keep at -20° C for long-term storage. Avoid repeated freezing and thawing.

Stability

When stored as instructed, expires one year from date of receipt unless otherwise indicated on the Certificate of Analysis.



Figure 1 Peptide Competition.

Extracts from CHO-T cells transiently transfected with wildtype human IRS1 that were unstimulated (lane 1) or stimulated with 50 nM insulin for 5 minutes (lanes 2–5) were resolved on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with 5% BSA-TBST for one hour at room temperature, then incubated with the IRS1 [pY612] antibody for 2 hrs at room temperature in 3% BSA-TBST, following prior incubation with: no peptide (lanes 1, 2), a non-phosphorylated peptide corresponding to the immunogen (lane 3), a generic phosphotyrosine-containing peptide (lane 4), or the phosphopeptide immunogen (lane 5). The blots were developed using chemiluminescence (ECL) method with a goat $F(ab')_2$ anti-rabbit IgG HRP conjugate (Cat. no. ALI4404).

Only the phosphopeptide corresponding to IRS1 [pY612] blocks the antibody signal (lane 5) demonstrating the specificity of the antibody.

Positive controls used

Insulin (50 nM for 5 minutes) or TPA (100 ng/mL for 30 min) treated human embryonic kidney cells (293T) or Chinese Hamster Ovary cells (CHO-T) transiently transfected with a plasmid encoding human IRS1.

Storage buffer

Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier, and 0.05% sodium azide.



CAUTION! Sodium azide is extremely toxic and may react with lead and copper plumbing to form highly explosive metal azides. Properly dispose of solutions containing sodium azide. Read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available at www.lifetechnologies.com/support.

References

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- Kaiser, C. and S.R. James (2004) Acetylation of insulin receptor substrate-1 is permissive for tyrosine phosphorylation. *BMC Biol.* 2(1):23-36.
- 3. Reynoso, R., et al. (2003) High levels of palmitic acid lead to insulin resistance due to changes in the level of phosphorylation of the insulin receptor and insulin receptor substrate-1. *Mol. Cell. Biochem.* 246(1-2):155-162.
- Hers, I., et al. (2002) Reciprocal feedback regulation of insulin receptor and insulin receptor substrate tyrosine phosphorylation by phosphoinositide 3-kinase in primary adipocytes. *Biochem. J.* 368(Pt 3):875-884.
- 5. Shao, J., et al. (2002) Phosphatidylinositol 3-kinase redistribution is associated with skeletal muscle insulin resistance in gestational diabetes mellitus. *Diabetes*. 51(1):19-29.
- 6. Fujioka, T., et al. (2001) Further evidence for the involvement of insulin receptor substrates in epidermal growth factor-induced activation of phosphatidylinositol 3-kinase. *Eur. J. Biochem.* 268(15):4158-4168.
- Shaw, L.M. (2001) Identification of insulin receptor substrate 1 (IRS-1) and IRS-2 as signaling intermediates in the alpha6 beta4 integrin-dependent activation of phosphoinositide 3-OH kinase and promotion of invasion. *Mol. Cell. Biol.* 21(15):5082-5093.
- 8. Esposito, D.L., et al. (2001) Tyr(612) and Tyr(632) in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of GLUT4 in adipose cells. *Endocrinology*. 142(7):2833-2840.

Related products

Product Name	Quantity	Cat. No.
IRS1 [pS616] Rabbit Polyclonal Antibody	10 blots	44550G
IR [pY972] Polyclonal Antibody, Rabbit	10 blots	44800G
IR/IGF1R [pYpYpY1158/1162/1163] Rabbit Polyclonal Antibody	10 blots	44806G
IRS1 [pS312] Rabbit Polyclonal Antibody	10 blots	44814G
IRS1 [pY896] Rabbit Polyclonal Antibody	10 blots	44818G
IRS1 [pY941] Rabbit Polyclonal Antibody	10 blots	44820G
IRS1 [pY1179] Rabbit Polyclonal Antibody	10 blots	44822G
IR (β-subunit) Human ELISA Kit	96 assays	KHR9111
IR [pY1158] Human ELISA Kit	96 assays	KHR9121
IR [pYpY1162/1163] Human ELISA Kit	96 assays	KHR9131
IR [pYpY1162/1163] Singleplex Bead Kit	100 assays	LHR9131

Product documentation

To obtain a Certificate of Analysis or Safety Data Sheets (SDSs), visit **www.lifetechnologies.com/support.**

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Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description		
***	Manufacturer	REF	Catalog number	LOT	Batch code		
	Use by	X	Temperature limitation				
i	Consult instructions for use	\triangle	Caution, consult accompanying documents				

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