

## Rabbit (polyclonal) Anti-IRS-2 (pS<sup>731</sup>) Phosphospecific Antibody, Unconjugated

## PRODUCT ANALYSIS SHEET

Catalog Number: 44-828

Lot Number: See product label

Quantity/Volume: See product label

**Form of Antibody:** Purified rabbit polyclonal immunoglobulin in phosphate buffered saline, pH 7.4.

**Preservation:** 0.02% sodium azide (Caution: sodium azide is a poisonous and hazardous substance.

Handle with care and dispose of properly.)

**Purification:** Purified from rabbit serum by epitope affinity chromatography.

Immunogen: This antibody was produced against a chemically synthesized phosphopeptide derived

from the human IRS-2 sequence, corresponding to the serine 731 phosphorylation site.

This site corresponds to serine 133 in mouse, and serine 233 in rat.

Specificity: Insulin exerts its effects by binding to the insulin receptor, a disulfide-linked

heterotetrameric protein comprised of two  $\alpha$  subunits and two  $\beta$  subunits arranged in the following configuration:  $\beta$ - $\alpha$ - $\alpha$ - $\beta$ . The  $\alpha$  subunits each contain insulin binding sites and are entirely extracellular in localization. The  $\beta$  subunits each possess an extracellular domain, a single transmembrane domain, and a cytoplasmic tyrosine kinase domain. Binding of insulin to the  $\alpha$  subunits induces a conformation change in which activates the kinase domain, stimulating autophosphorylation of the receptor and tyrosine phosphorylation of at least five different insulin receptor substrates designated IRS-1-4, and Shc. This tyrosine phosphorylation produces docking sites for proteins bearing SH2 domains, such as PI3-K, Grb-2, Nck, and Crk. While tyrosine phosphorylation of insulin receptor substrates propagates signaling from insulin, serine and threonine phosphorylation by MAPK's, PKA, and PKC may reduce insulin signaling. This antibody recognizes IRS-2

when phosphorylated at serine 731.

**Species Reactivity:** Human, mouse, and rat. Other species were not tested.

**Applications:** This antibody is suitable for use in ELISA, Western blotting, and immunoprecipitation.

**Suggested Working** 

Dilutions:

The recommended concentration for use in immunoprecipitation is 3-5  $\mu$ g/extract from  $10^7$  cells; for Western blotting, 0.5-2.0  $\mu$ g/mL; and for ELISA, 0.1-1.0  $\mu$ g/mL. The

optimal concentration should be determined for each specific application.

**Recommended Positive** 

**Control:** 

Serum-treated mouse fibroblast (3T3) cells.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 509, 681

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.

(Rev 11/08) DCC-08-1089

Storage:

Store at 2-8°C for up to one month. For long term storage, apportion into working aliquots and store at -20°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.

**Expiration Date:** 

Expires one year from date of receipt when stored as instructed.

**References:** 

Greene, M.W., H. Sakaue, L. Wang, D.R. Alessi, and D.A. Roth (2003) Modulation of insulin stimulated degradation of human insulin receptor substrate-1 by serine 312 phosphorylation. J. Biol. Chem. 278:8199-8211.

Sun, X.J., P. Rothenberg, C.R. Kahn, J.M. Backer, E. Araki, P.A. Wilden, D.A. Cahill, B.J. Goldstein, and M.F. White (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352:73-77.

Sun, X.J., L.M. Wang, Y. Zhang, L. Yenush, M.G. Myers, Jr, E. Glasheen, W.S. Lane, J.H. Pierce, and M.F. White (1995) Role of IRS-2 in insulin and cytokine signalling. Nature 377:173-177.

Mothe I., and E. van Obberghen (1996) Phosphorylation of insulin receptor substrate-1 on multiple serine residues, 612, 632, 662, and 731, modulates insulin action. J. Biol. Chem. 271:11222-11227.

Lavan, B.E., W.S. Lane, and G.E. Lienhard (1997) The 60-kDa phosphotyrosine protein in insulin-treated adipocytes is a new member of the insulin receptor substrate family. J. Biol. Chem. 272:11439-11443.

Lavan, B.E., V.R. Fantin, E.T. Chang, W.S. Lane, S.R. Keller, and G.E. Lienhard (1997) A novel 160-kDa phosphotyrosine protein in insulin-treated embryonic kidney cells is a new member of the insulin receptor substrate family. J. Biol. Chem. 272:21403-21407.

White, M.F. (1998) The IRS-signalling system: a network of docking proteins that mediate insulin action. Mol. Cell. Biochem. 182(1-2):3-11 (Review).

Liu, Y.F., K. Paz, A. Herschkovitz, A. Alt, T. Tennenbaum, S.R. Sampson, M. Ohba, T. Kuroki, D. LeRoith, and Y. Zick (2001) Insulin stimulates PKCzeta -mediated phosphorylation of insulin receptor substrate-1 (IRS-1). A self-attenuated mechanism to negatively regulate the function of IRS proteins. J. Biol. Chem. 276:14459-14465.