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Product Information

Anti-Insulin Receptor Substrate-1 (IRS-1)
produced in rabbit, affinity isolated antibody

Catalog Number **I7153**

Product Description

Anti-Insulin Receptor Substrate 1 (IRS-1) is produced in rabbit using the C-terminal 14 amino acid residues of rat liver IRS-1 as immunogen.

Anti-IRS-1 specifically recognizes the insulin receptor substrate 1 (165 kDa) by immunoblotting. The antibody shows species cross reactivity with human, rat, and mouse IRS-1. The antibody may also be used for immunoprecipitation.

Insulin receptor substrate 1 (IRS-1) is a major effector of insulin receptor action. IRS-1 is an adapter protein that binds the activated insulin receptor and is phosphorylated on multiple tyrosine residues. Phosphorylated IRS-1 binds cytoplasmic signaling proteins containing SH2 domains and in turn helps receptor mediated signal transduction.[1]

The Insulin receptor is a transmembrane protein, which consists of 4 subunits ($2\alpha2\beta$) and exhibits tyrosine kinase activity. Upon binding of insulin to the extracellular subunit, the 95kDa subunit of tyrosine kinase is activated. Receptor-mediated phosphorylation of the insulin receptor substrate proteins is needed to begin signaling of processes such as glucose transport and mitogenesis.

Reagent

Supplied as a solution in 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, containing 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –20 °C. Aliquot to avoid repeated freezing and thawing. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedures

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 mg/ml total cell protein in a microcentrifuge tube with PBS, Catalog Number P3813.
2. Add 4 µg of Anti-IRS-1 to 0.5-1 mg cell lysate.
3. Gently rock the reaction mixture at 2-8 °C overnight.
4. Capture the immunocomplex by adding 100 µL of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads, Catalog Number P2545, 50 µl packed beads.
5. Gently rock reaction mixture at 2-8 °C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x *g*), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µL 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na_3VO_4, and 1 mM NaF.

Product Profile

Immunoblotting: a working concentration of 0.5-2 µg/ml is recommended using 3T3/A31 mouse fibroblasts in RIPA cell lysates.

Immunoprecipitation: 4 µg recommended to immunoprecipitate IRS-1 from 0.5 mg of a mouse 3T3/A31 RIPA cell lysate.

Note: In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

1. Myers, M.G., et al., *Endocrinology*, **131**, 2196 (1992).
2. Myers, M.G. Jr., and White, M.F., *Ann. Rev. Pharmacol. Toxicol*., **36**, 615 (1996).
3. Waters, S.B., et al., *J. Biol. Chem*., **268**, 22231 (1993).

BKR,PHC 12/09-1

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