Anti-Insulin Receptor Substrate 2
produced in rabbit, IgG fraction of antiserum

Catalog Number I5034

Product Description
Anti-Insulin Receptor Substrate 2 is produced in rabbit using as immunogen a GST-fusion protein containing the amino acid residues 976-1094 of mouse insulin receptor substrate 2 (IRS-2). It is IgG immunoaffinity purified.

Anti-Insulin Receptor Substrate 2 (IRS-2) specifically recognizes insulin receptor substrate 2 (170-185 kDa). Other weaker, lower molecular weight bands may also be observed. The antibody shows species cross-reactivity with mouse, rat, and human IRS-2. Reactivity with other species has not been confirmed. The antibody may be used in immunoblotting and 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. IRS-2-deficient mice display all characteristics of type 2 diabetes, suggesting that dysfunction of the IRS-2 gene may contribute to the pathogenesis of human type 2 diabetes. IRS-1 and IRS-2 are critical for embryonic and post-natal growth, with IRS-1 having the predominant role. By contrast, both IRS-1 and IRS-2 function in peripheral carbohydrate metabolism, but IRS-2 has the major role in β-cell development and compensation for peripheral insulin resistance.

The insulin receptor is a transmembrane protein, which consists of 4 subunits (2α2β) and exhibits tyrosine kinase activity. Upon binding of insulin to the extracellular subunit, the 95 kDa 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 in 20 mM phosphate buffer, pH 7.6, 0.25 M NaCl, and 0.1% 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. Do not store in a frost-free freezer. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure – 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 µL of the antibody to 60 µL (30 µL packed beads) of washed Protein A agarose bead slurry, Catalog Number P2545; add to 500 µL of phosphate buffered saline in a microcentrifuge tube. Gently rock the reaction mixture at 4 °C for 1 hour.
3. 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 phosphate buffered saline.
4. Dilute the cell lysate to ~1 µg/µL total cell protein with phosphate buffered saline.
5. Add 100 µg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4 °C for 1 hour.
7. 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 phosphate buffered saline.
8. Resuspend the agarose beads in 60 µL 2X Laemmli sample buffer. The agarose beads can be frozen for later use, or boil the beads for 5 minutes.

9. After boiling, 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, and pepstatin, 1 mM Na$_3$VO$_4$, and 1 mM NaF.

Product Profile
Immunoblotting: a working concentration of 1:500-1:2,000 is recommended using mouse 3T3/A31 RIPA cell lysates in a chemiluminescence detection system. Lysates of human A431 cells and rat L6 cells may also be used to detect IRS-2.

Immunoprecipitation: 4 µL is recommended to immunoprecipitate IRS-2 from 0.1 mg of a mouse 3T3/A31 RIPA 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