ANTI-INSULIN RECEPTOR, β-SUBUNIT
Developed in Rabbit,
IgG Fraction of Antiserum

Product Number I 6153

Product Description
Anti-Insulin Receptor, β-subunit is developed in rabbit using a GST-fusion protein corresponding to the last 100 C-terminal amino acid residues of the beta subunit of the human insulin receptor as immunogen. The antibody is purified using protein A.

Anti-Insulin Receptor, β-subunit specifically recognizes the β-subunit of the human insulin receptor (~97 kD) by immunoblotting. In some preparations, the antibody may recognize a higher M.W. band (the insulin receptor precursor) and a lower m.w. band. There is no cross-reactivity with human IGF-1 receptor. Species cross-reactivity include human and rat.

Anti-Insulin Receptor, β-subunit may be used for immunoblotting, immunoprecipitation and immunocytochemistry.

The insulin receptor is a transmembrane protein which consists of 4 subunits (2α2β) and exhibits receptor tyrosine kinase activity (RTK). RTKs are single-pass transmembrane receptors that possess intrinsic cytoplasmic enzymatic activity, catalyzing the transfer of the gamma-phosphate of ATP to tyrosine residues in protein substrates. RTKs are essential components of signal transduction pathways that affect cell proliferation, differentiation, migration and metabolism. Included in this large protein family are the insulin receptor and the receptors for growth factors such as epidermal growth factor, fibroblast growth factor and vascular endothelial growth factor receptor. Receptor activation occurs through ligand binding, which facilitates receptor dimerization and autophosphorylation of specific tyrosine residues in the cytoplasmic portion. The interaction of insulin with the α-subunit of the insulin receptor activates the protein tyrosine kinase of the β-subunit, which then undergoes an autophosphorylation that increases its tyrosine kinase activity. Three adapter proteins, IRS-1, IRS-2 and Shc, become phosphorylated on tyrosine residues following insulin receptor activation. These three phosphorylated proteins then interact with SH2 domain-containing signaling proteins.

Insulin is a 51-amino acid polypeptide composed of A and B chains connected by disulfide bonds. Its precursor, proinsulin, is a single chain molecule consisting of A and B chains connected through the C-peptide. Proinsulin, which has little biological activity, is cleaved by proteases within its cell of origin into the insulin molecule and the C-terminal basic residue. The main storage sites for insulin and the C-peptide are the pancreatic islets. Insulin is one of the major regulatory hormones of intermediate metabolism throughout the body. The biological actions of this hormone involve integration of carbohydrate, protein, and lipid metabolism. Insulin enhances membrane transport of glucose, amino acids, and certain ions. It also promotes glycogen storage, formation of triglycerides and synthesis of protein and nucleic acids. The exact mechanism(s) by which insulin achieves these intracellular effects remains somewhat elusive, but is assumed to involve activation of one or more second messengers. Immunocytochemical investigations have localized insulin in the B or β-cells of pancreatic islets of Langerhans. These cells, characterized for many years on the basis of their histochemical and ultrastructural features, comprise 70-80% of the pancreatic islet cells, and are located toward the center of the islets. The hormone is stored inside secretory granules which possess a crystalline core displaying a well-defined periodicity by electron microscopy. Deficiency of insulin results in diabetes mellitus, one of the leading causes of death in the general population. Insulin is also present in tumors of β-cell origin such as insulinoma. Insulin-specific antibodies prove useful as β-cell and tumor markers using immunohistochemical techniques, and as analytical tools in quantification of the hormone.

Reagents
Anti-Insulin Receptor, β-subunit is supplied as purified IgG fraction in 0.1M tris-glycine, pH 7.4, containing 0.15 M NaCl and 0.05% sodium azide.

Protein concentration is approximately 1 mg/ml by biuret.
Storage/Stability
For continuous use, store at 2 °C -8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedures
1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 mg/ml total cell protein in a microcentrifuge tube with PBS (Product No. P 3813).
2. Add 5 µg of Anti-Insulin Receptor, β-subunit to 0.5-1 mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Product No. P 2545).
5. Gently rock reaction mixture at 4 °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.

Immunocytochemistry
1. Plate approximately 200 µl of a cell suspension into each well of a slide. Incubate 24 hours in a 37 °C CO₂ incubators.
2. Wash the cells 3 X for 5 min. with PBS. Do not shake cells.
3. Add fixative (ice-cold 4% paraformaldehyde in PBS) for 20 min. at room temperature.
4. Wash the cells with PBS, 2 X for 15 min. with gentle agitation. Do not shake cells.
5. Add 400 µl PBS containing 8% BSA and incubate 30 minutes at room temperature.
6. Wash cells 2 X with PBS for 15 min.
7. Incubate the cells with 10 µg/ml of Anti-Insulin Receptor, β-subunit in PBS containing 1% BSA and incubate overnight at 4 °C.
8. Wash the cells 2 X with PBS for 15 min.
9. Incubate the cells with a 1:150 dilution of anti-rabbit IgG conjugated with FITC (Product No. F 9887) in PBS for 1 hr. at room temperature in the dark.
10. Wash the cells 3 X with PBS for 15 min. in the dark.
11. Mount coverslips with gel mount and allow gel mount to dry in the dark.
12. Examine the cells under a fluorescent microscope.

Product Profile
A working concentration of 1-4 µg/ml is recommended for immunoblotting using RIPA cell lysates of mouse 3T3 transfected with human insulin receptor.

For immunoprecipitation, 5 µg is recommended to immunoprecipitate insulin receptor from 0.5 mg of a RIPA cell lysates of mouse 3T3 transfected with human insulin receptor.

A working concentration of 10 µg/ml is recommended for immunocytochemistry using 4% paraformaldehyde-fixed mouse 3T3 cells transfected with human insulin receptor.

Note 1: Due to the limited numbers of receptors, concentration of receptor preparations with WGA-agarose (Product No. L 1394) is recommended.

Note 2: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References