# Anti-Insulin Receptor Beta Antibody [SN20-13] ET1611-90

Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IF-Cell, IF-Tissue, IHC

Molecular Wt: Predicted band size: 156 kDa

Clone number: SN20-13

**Description:** The insulin receptor (IR) is a heterodimeric protein complex that has an intracellular  $\beta$ 

subunit and an extracellular  $\alpha$  subunit, which is disulfide- linked to a transmembrane segment. The insulin ligand binds to the IR and initiates molecular signaling pathways that promote glucose uptake in cells and glycogen synthesis. Insulin binding to IR induces phosphorylation of intracellular tyrosine kinase domains and recruitment of multiple SH2 and SH3 domain-containing intracellular proteins that serve as signaling intermediates for pleiotropic effects of insulin. The human insulin receptor gene maps to chromosome 19p13.3-p13.2 and encodes a 1382 amino acid protein that cleaves to form  $\alpha$  and  $\beta$  subunits. Type 1 diabetes is an auto-immune condition of the endocrine pancreas that results in destruction of insulin secreting cells and a progressive loss in insulin-sensitive glucose uptake by cells.

Type 2 diabetes is a condition where cells become resistant to insulin action.

**Immunogen:** Synthetic peptide within human Insulin receptor aa 930-970.

Positive control: HCT 116 cell lysate, MDA-MB-231 cell lysate, HepG2 cell lysate, T-47D cell lysate, Rat liver

tissue lysate, mouse skeletal muscle tissue lysates, HepG2, LO2, RH-35, rat liver tissue,

mouse liver tissue, human liver tissue.

Subcellular location: Cell membrane.

Database links: SwissProt: P06213 Human | P15208 Mouse | P15127 Rat

Recommended Dilutions:

WB 1:500-1:2,000
IF-Cell 1:100-1:500
IF-Tissue 1:100-1:500
IHC 1:1,000

Storage Buffer: 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at  $4^{\circ}$ C. Store at  $+4^{\circ}$ C short term (1-2 weeks). It is recommended to aliquot into

single-use upon delivery. Store at -20 °C long term.

**Purity:** Protein A affinity purified.

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#### **Images**

kDa 250-150-150-100-75-55-45-35-25-HSP90 Fig1: Western blot analysis of Insulin Receptor Beta on different lysates with Rabbit anti-Insulin Receptor Beta antibody (ET1611-90) at 1/1,000 dilution.

Lane 1: HCT 116-si NT cell lysate

Lane 2: HCT 116-si Insulin Receptor Beta cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 156 kDa Observed band size: 95 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Fig2: Western blot analysis of Insulin Receptor Beta on different lysates with Rabbit anti-Insulin Receptor Beta antibody (ET1611-90) at 1/1,000 dilution.

Lane 1: MDA-MB-231 cell lysate (20 µg/Lane)

Lane 2: HepG2 cell lysate (20 µg/Lane)

Lane 3: T-47D cell lysate (low expression) (20 µg/Lane)

Lane 4: Hepa1-6 cell lysate (20 µg/Lane)

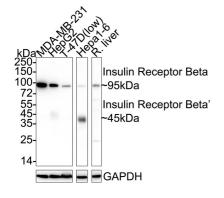
Lane 5: Rat liver tissue lysate (40 µg/Lane)

Predicted band size: 156 kDa Observed band size: 95/45 kDa

Exposure time: Lane 1-3: 1 minute 2 seconds; Lane 4-5: 3

minutes; ECL: K1802;

4-20% SDS-PAGE gel.



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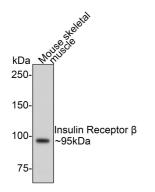


Fig3: Western blot analysis of Insulin Receptor Beta on mouse skeletal muscle tissue lysates with Rabbit anti-Insulin Receptor Beta antibody (ET1611-90) at 1/500 dilution.

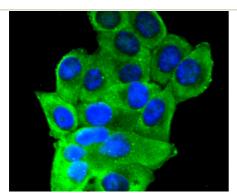
Lysates/proteins at 20 µg/Lane.

Predicted band size: 156 kDa Observed band size: 95 kDa

Exposure time: 3 minutes;

4~20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1611-90) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.



**Fig4:** ICC staining of Insulin Receptor Beta in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-90, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

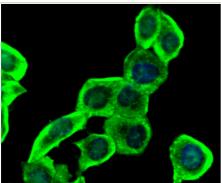
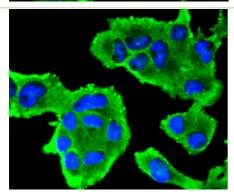


Fig5: ICC staining of Insulin Receptor Beta in LO2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-90, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig6:** ICC staining of Insulin Receptor Beta in RH-35 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1611-90, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

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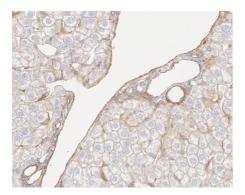
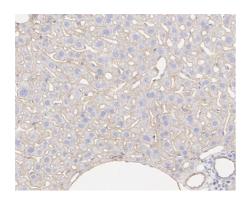


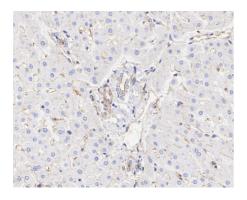
Fig7: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Insulin Receptor Beta antibody (ET1611-90) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1611-90) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig8:** Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Insulin Receptor Beta antibody (ET1611-90) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1611-90) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig9:** Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Insulin Receptor Beta antibody (ET1611-90) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1611-90) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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### **Background References**

- 1. Cao, L. et al. 2012. Hepatic insulin signaling changes: possible mechanism in prenatal hypoxia-increased susceptibility of fatty liver in adulthood. Endocrinology. 153: 4955-65.
- 2. Ruttenstock, E. et al. 2011. Prenatal administration of retinoic acid upregulates insulin-like growth factor receptors in the nitrofen-induced hypoplastic lung. Birth Defects Res. B Dev. Reprod. Toxicol.. 92: 148-151.