Species Reactivity: Human/Mouse
Specificity: Detects human IGF-I R/IGF1R in sandwich ELISAs and Western blots. Detects mouse IGF-I R/IGF1R in Immunohistochemistry. In sandwich immunoassays, less than 0.15% cross-reactivity or interference was observed with recombinant human (rh) IGF-I, rhIGF-II, rhIL-3 Ra, rhIL-9 R, and rhTGF-β RII.
Source: Monoclonal Mouse IgG1 Clone # 33255
Purification: Protein A or G purified from hybridoma culture supernatant
Immunogen: S. frugiperda insect ovarian cell line Sf 21-derived recombinant human IGF-I R/IGF1R Glu31-Asn932 Accession # P08069
Endotoxin Level: <0.10 EU per 1 μg of the antibody by the LAL method.
Formulation: Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

*Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS

Recommended Concentration

<table>
<thead>
<tr>
<th>Sample</th>
<th>Western Blot</th>
<th>Flow Cytometry</th>
<th>Immunocytochemistry</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 µg/mL</td>
<td>0.25 µg/10⁶ cells</td>
<td>3-25 µg/mL</td>
<td>5-25 µg/mL</td>
</tr>
</tbody>
</table>

**Human IGF-I R/IGF1R Sandwich Immunoassay**

Reagent

- ELISA Capture: 2-8 µg/mL Human/Mouse IGF-I R/IGF1R Antibody (Catalog # MAB391)
- ELISA Detection: 0.1-0.4 µg/mL Human IGF-I R/IGF1R Biotinylated Antibody (Catalog # BAF391)

Neutralization

Measured by its ability to neutralize IGF-I-induced proliferation in the MCF-7 human breast cancer cell line. Karey, K.P. et al. (1988) Cancer Research 48:4083. At 11 µg/mL, this antibody will neutralize approximately 50-75% of the bioactivity due to 6 ng/mL Recombinant Human IGF-I.

**DATA**

**Western Blot**

Detection of Human IGF-I R/IGF1R by Western Blot. Western blot shows lysates of NTERa-2 human testicular embryonic carcinoma cell line, SK/Mel-28 human malignant melanoma cell line, and G361 human melanoma cell line. PVDF membrane was probed with 1 µg/mL of Mouse Anti-Human/Mouse IGF-I R/IGF1R Monoclonal Antibody (Catalog # MAB391) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for IGF-I R/IGF1R at approximately 275 kDa (as indicated). This experiment was conducted under non-reducing conditions and using Immunoblot Buffer Group 2.

**Flow Cytometry**

Detection of IGF-I R/IGF1R in MCF-7 Human Cell Line by Flow Cytometry. MCF-7 human breast cancer cell line was stained with Mouse Anti-Human IGF-I R/IGF1R Monoclonal Antibody (Catalog # MAB391, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram) followed by anti-mouse IgG PE-conjugated secondary antibody (Catalog # F0102B). View our protocol for Staining Membrane-associated Proteins.
Immunocytochemistry

IGF-I R/IGF1R in MCF-7 and HDLM Human Cell Lines. IGF-I R/IGF1R was detected in immersion fixed MCF-7 human breast cancer cell line (positive staining; left panel) and HDLM human Hodgkin’s lymphoma cell line (negative staining; right panel) using Mouse Anti-Human/Mouse IGF-I R/IGF1R Monoclonal Antibody (Catalog # MAB391) at 3 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell membrane in MCF-7 cell line. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry

IGF-I R/IGF1R in Mouse Heart. IGF-I R/IGF1R was detected in perfusion fixed paraffin-embedded sections of mouse heart using Mouse Anti-Human/Mouse IGF-I R/IGF1R Monoclonal Antibody (Catalog # MAB391) at 15 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membrane and cytoplasm. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Neutralization

Neutralization of Cell Proliferation Induced by IGF-I and Neutralization by Human IGF-I R/IGF1R Antibody. Recombinant Human (rh) IGF-I (Catalog # 291-G1) stimulates proliferation in the MCF-7 human breast cancer cell line in a dose-dependent manner (orange line). Proliferation elicited by rhIGF-I (6 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human/Mouse IGF-I R/IGF1R Monoclonal Antibody (Catalog # MAB391). At 11 µg/mL, this antibody will neutralize 50-75% rhIGF-1 induced activity.

PREPARATION AND STORAGE

Reconstitution

Reconstitute at 0.5 mg/mL in sterile PBS.

Shipping

The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

Stability & Storage

- Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Insulin-like growth factor I receptor (IGF-I R) is a disulfide-linked heterotetrameric transmembrane protein consisting of two α and two β subunits. Both the α and β subunits are encoded within a single receptor precursor CDNA. The proreceptor poly peptide is proteinlytically cleaved and disulfide-linked to yield the mature heterotetrameric receptor. The α subunit of IGF-I R is extracellular while the β subunit has an extracellular domain, a transmembrane domain and a cytoplasmic tyrosine kinase domain. IGF-I R is highly expressed in all cell types and tissues. Essentially all of the biological activities of IGF-I and -II have been shown to be mediated via IGF-I R.