

## ORDERING INFORMATION

**Catalog Number:** MAB391

**Clone:** 33255

**Lot Number:** YY07

**Size:** 500 µg

**Formulation:** 0.2 µm filtered solution in PBS with 5% trehalose

**Storage:** -20° C

**Reconstitution:** sterile PBS

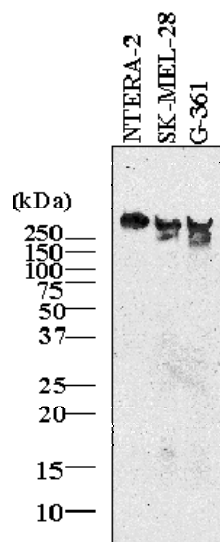
**Specificity:** human IGF-I R

**Immunogen:** Sf21-derived rhIGF-I R

**Ig class:** mouse IgG<sub>1</sub>

### Recommended Applications:

Neutralization of bioactivity  
Western blot  
ELISA capture



**Figure 1**  
**Detection of IGF-I R with MAB391.**

Lysates were prepared from NTERA-2 human embryonal carcinoma cells, SK-MEL-28 human malignant melanoma cells, and G-361 human malignant melanoma cells in non-reducing sample buffer. The lysates were resolved by SDS-PAGE and transferred to an Immobilon-P membrane. The blot was developed with 1 µg/mL monoclonal anti-human IGF-I R antibody and Chemiluminescent Detection reagent.

## Background

Insulin-like growth factor I receptor (IGF-I R) is a disulfide-linked heterotetrameric transmembrane protein consisting of two  $\alpha$  and two  $\beta$  subunits. Both the  $\alpha$  and  $\beta$  subunits are encoded within a single receptor precursor cDNA. The proreceptor polypeptide is proteolytically cleaved and disulfide-linked to yield the mature heterotetrameric receptor. The  $\alpha$  subunit of IGF-I receptor is extracellular while the  $\beta$  subunit has an extracellular domain, a transmembrane domain and a cytoplasmic tyrosine kinase domain. The IGF-I receptor 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.

## Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, insect cell line Sf21-derived, recombinant human IGF-I R extracellular domain (rhIGF-I R; aa 31 - 932; Accession # P08069). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

## Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Endotoxin Level

< 0.1 EU per 1 µg of the antibody as determined by the LAL method.

## Reconstitution

Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 µg/mL.

## Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

## Specificity

This antibody was selected for its ability to block human IGF-I R mediated bioactivities induced by IGF-I or IGF-II and for use as a capture antibody in human IGF-I R sandwich ELISAs. When used in combination with the biotinylated anti-human IGF-I R detection antibody (Catalog # BAF391) in sandwich ELISAs, less than 0.15% cross-reactivity was observed with rhIGF-I, rhIGF-II, rhIL-3 R $\alpha$ , rhIL-9 R and rhTGF- $\beta$  RII.

## Applications

**Neutralization of Human IGF-I receptor-mediated bioactivity** - The exact concentration of antibody required to neutralize the human IGF-I R mediated bioactivity is dependent on the IGF-I or IGF-II concentration and on the number and types of IGF-I receptors present on the cell surface (a function of cell type and culture conditions). To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization Dose<sub>50</sub> (ND<sub>50</sub>)** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cell surface IGF-I R mediated IGF response on a responsive cell line, at a specific IGF concentration.

At 11 µg/mL, this anti-human IGF-I R antibody will neutralize approximately 50 - 75% of IGF-I R-mediated rhIGF-I biological activity on MCF-7 cells. The specific conditions are described in the figure legends.

**Western blot** - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human IGF-I R. Using a colorimetric detection system, the detection limit for rhIGF-I R is approximately 25 ng/lane under non-reducing conditions. Chemiluminescent detection will increase sensitivity by 5 to 50 fold. Refer to [www.RnDSystems.com/WBCellLysates](http://www.RnDSystems.com/WBCellLysates) for detailed procedures for preparing lysates and Western blotting. The specific buffers are listed on the next page.

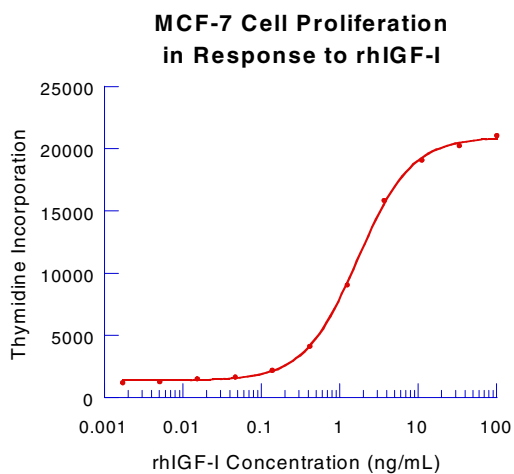
## Protocols for Immunoblotting

### Western blotting

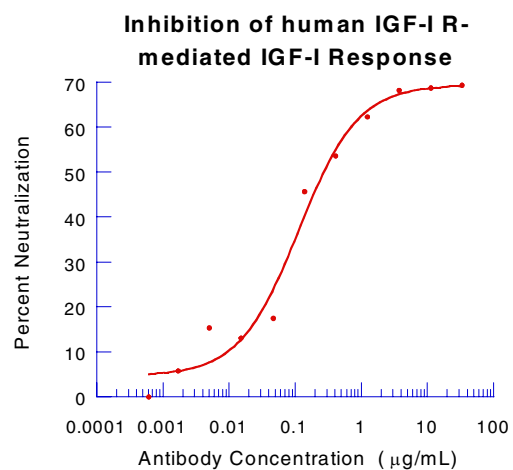
<u>Wash Buffer</u>	<u>Blocking Buffer</u>	<u>Blotting Buffer</u>
25 mM Tris, pH 7.4	5% nonfat dry milk	2% nonfat dry milk
0.15 M NaCl	in Wash Buffer	in Wash Buffer
0.05% Tween® 20		

**ELISA capture** -This product can be used as a capture reagent in a human IGF-I R sandwich immunoassay in combination with biotinylated, human IGF-I R detection antibody (Cat. # BAF391) and recombinant human IGF-I R, (Cat. # 391-GR) as the standard. The suggested coating concentration range is 2 - 8 µg/mL and should be titrated to determine the optimal concentration. A general protocol is provided at [www.RnDSystems.com/go/MAPELISA](http://www.RnDSystems.com/go/MAPELISA).

**Figure 2**



**Figure 3**



**Figure 2**

Human IGF-I stimulates the proliferation of human MCF-7 cells in a dose-dependent manner (Karey, K.P. *et al.*, 1988, *Cancer Research* **48**:4083). The ED<sub>50</sub> for this effect is typically 1 - 3 ng/mL.

**Figure 3**

To measure the ability of the antibody to block the IGF-I R mediated IGF response on MCF-7 cells, cells were added to wells in a 96 well plate containing the various concentrations of antibody and preincubated for 1 hour at 37° C. Following this preincubation, rhIGF-I is added. The assay mixture in a total volume of 100 µL, containing antibody at the concentrations indicated, rhIGF-I at 6.0 ng/mL and cells at 5 x 10<sup>4</sup> cells/mL, was incubated at 37° C for 72 hours in a humidified CO<sub>2</sub> incubator and pulsed with <sup>3</sup>H-thymidine for the final 24 hours. The cells are subsequently detached, harvested onto glass fiber filters and the <sup>3</sup>H-thymidine incorporated into DNA was determined. Approximately 11 µg/mL of the antibody will neutralize 50 - 75% of the bioactivity due to 6 ng/mL of rhIGF-I.