



cell signaling solutions

## Certificate of Analysis

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### Anti-IGF-1 R, clone JBW902

(mouse monoclonal IgG<sub>2a</sub>)

Catalog # 05-656

Lot # 22215

**Immunogen:** GST fusion protein corresponding to residues 1264-1367 of human IGF-1 Receptor.

**Specificity:** IGF-1 receptor  $\beta$  chain, MW 95kDa. Does not cross react with insulin receptor.

**Species Cross-reactivity:** Human, mouse.

**Formulation:** 200 $\mu$ g of protein A purified mouse IgG<sub>2a</sub> in 200 $\mu$ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 8.4%. Liquid at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

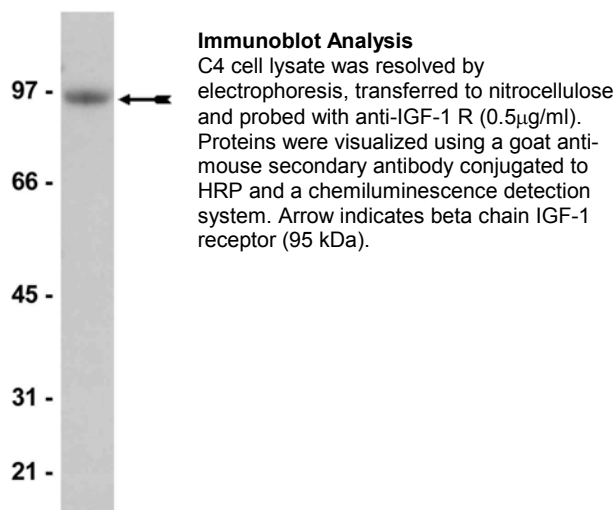
**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

### Quality Control Testing and Research Applications

Since the number of IGF-1 receptors is limited, subfractionation by WGA-agarose chromatography can be used to enrich receptor number prior to use.<sup>1,2</sup>

**Immunoblot Analysis:** 0.1-2 $\mu$ g/ml of this lot detected the beta chain of IGF-1 Receptor in RIPA lysates from C4 cells (mouse 3T3 cells that have been transfected with the human IGF-1 Receptor) and in RIPA lysates from mouse 3T3/A31 cells.

**Immunoprecipitation:** 4 $\mu$ g/ml of this lot immunoprecipitated IGF-1 Receptor from 1mg of RIPA lysates from C4 cells (mouse 3T3 cells that have been transfected with the human IGF-1 Receptor) and in RIPA lysates from mouse 3T3/A31 cells.



#### References:

1. Rosenzweig, S.A., *et al.*, *J. Biol. Chem.* **265**: 18030-18034, 1990.
2. Oemar, B.S., *et al.*, *J. Biol. Chem.* **266**: 2369-2373, 1991.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a C4 cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 10% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.1-2 $\mu$ g/ml of anti-IGF-1 R**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution was used) in TBST-MLK for 1 hour at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

### Immunoprecipitation Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1 $\mu$ g/ $\mu$ l total cell protein in a microcentrifuge tube with PBS.
2. Add **4 $\mu$ g of anti-IGF1 R** to 500 $\mu$ g-1mg cell lysate.
3. Gently rock the reaction mixture at room temperature for 2 hours
4. Capture the immunocomplex by adding 60 $\mu$ l (30 $\mu$ l packed beads) of washed Protein A agarose bead slurry, Catalog # 16-125.
5. Gently rock the reaction mixture at room temperature for 1 hour.
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 PBS.
7. Suspend the agarose beads in 60 $\mu$ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.