

Certificate of Analysis

Anti-IGF-1 R, clone JBW902
 (mouse monoclonal IgG_{2a})
 Catalog # 05-656
 Lot # DAM1564511

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

Specificity: IGF-1 receptor β chain, MW 95kDa. Does not cross react with insulin receptor.

Species Cross-reactivity: Human, mouse.

Formulation: 200 μ g of protein A purified mouse IgG_{2a} in 200 μ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide.

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**

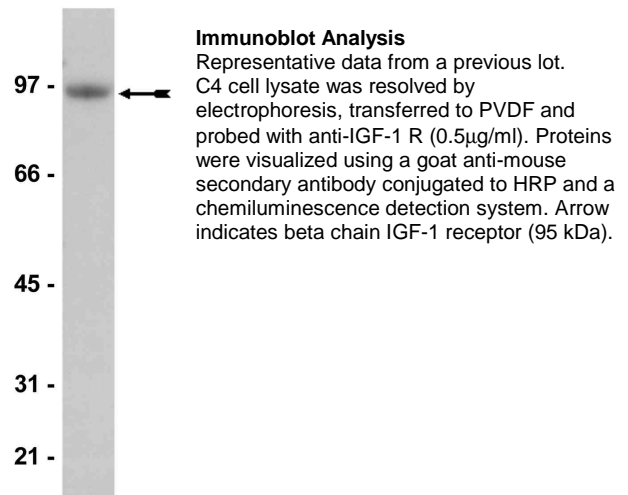
Quality Control Testing and Research Applications

Representative data from previous tested lot.

Since the number of IGF-I receptors is limited, subfractionation by WGA-agarose chromatography can be used to enrich receptor number prior to use.^{1,2}

Immunoblot Analysis: 0.1-2 μ 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 μ 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; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to PVDF. Wash the blotted PVDF twice with water.
2. Block the blotted PVDF 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 PVDF with **0.1-2µg/ml of anti-IGF-1 R**, diluted in freshly prepared TBST-MLK overnight with agitation at 4°C.
4. Wash the PVDF twice with water.
5. Incubate the PVDF 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 PVDF with water twice.
7. Wash the PVDF in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the PVDF 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 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **4µg of anti-IGF1 R** to 500µg-1mg cell lysate.
3. Gently rock the reaction mixture at room temperature for 2 hours
4. Capture the immunocomplex by adding 60µl (30µl 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 60µl 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.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2007: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.