

Anti-Human Insulin-like Growth Factor I

(mouse monoclonal, IgG_{1κ})

Catalog # 05-172

Lot # 12066

Antibody Class: IgG_{1κ} antibody (sm 1.2) produced by P3x63Ag8.653x Balb/c mouse spleen cells. Propagated as mouse ascites and purified by ammonium sulfate precipitation.¹

Immunogen: Human insulin-like growth factor I (IGF-I) purified from human plasma.²

Specificity: Specific to IGF-I.¹ Approximately 40% cross reactivity to IGF-II.⁵

Species Cross-reactivity: Human, mouse, rat and possibly chick; occasional difficulty neutralizing rat IGF-I due to binding protein interference.

Formulation: 500µg of purified IgG_{1κ} lyophilized from 44µl of water.. Quantitated by spectrophotometry using the extinction coefficient for gamma globulin [E (1%, 280nm)] = 14. Lyophilized powder.

Sterility: Sterilized through a 0.2µ membrane filter and packaged aseptically.

Storage and Stability: Stable lyophilized for 1 year at -20°C from date of shipment. Rehydrate in 250µl of PBS, stable for 6 months at -20°C once rehydrated. Aliquot rehydrated solution to avoid repeated freezing and thawing.

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

Quality Control Testing and Research Applications

Western Immunoblot Analysis: 1-2µg/ml of this lot detected 100ng of IGF-I under non-reducing conditions.

Immunoprecipitation: 5µg of this lot immunoprecipitated 100ng IGF-I.

Immunohistochemistry: 10µg/ml. Reported by an independent laboratory to detect IGF-I in formalin-fixed, paraffin-embedded skin sections.⁶

Neutralization: 10-30µg/ml of this antibody can inhibit the activity of 20ng/ml of IGF-I as determined by Balb/c 3T3 cell growth¹.

References:

1. Russell, W.E., *et al.*, Proc. Natl. Acad. Sci. USA. **81**: 2389-2392, 1984.
2. van Wyk, J.J., *et al.*, Human Growth Hormone 585-599, Plenum Publishing Corp, NY 1986.
3. Gillespie, G.Y., *et al.*, Meth. Enz. **146**: 207-216, 1987.
4. Han, V.K., *et al.*, Science **236**: 193-197, 1987
5. van Wyk, J.J., *et al.*, Endocrinology **138**: 4521, 1997.
6. Lui, *et al.*, Molc. Cell. Neurosci. **5**: 418-429, 1994.

Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a non-reduced sample and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1µg/ml of a-Human Insulin-like Growth Factor I**, diluted in freshly prepared PBS-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** IgG linked to horseradish peroxidase, 1:3000 dilution, was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-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. Before beginning the immunoprecipitation, dilute the sample to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **5µg of a-Human Insulin-like Growth Factor I** to the sample.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl of washed Protein G agarose bead slurry (50µl packed beads).
5. Gently rock the reaction mixture at 4°C for 2 hours.
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 either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50µl 2X Laemmli sample buffer and boil for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant, or the agarose beads can then be frozen for later use and reboiled for 5 minutes prior to SDS-PAGE.

Immunohistochemistry

1. Fix 10µm frozen tissue sections in ice cold formalin for 30 minutes at room temperature.
2. Wash the sections with PBS three times, 5 minutes each, at room temperature.
3. Add 400µl of 8% albumin in PBS and incubate for 30 minutes at room temperature.
4. Wash the sections with PBS three times, 5 minutes each, at room temperature.
5. Incubate the sections with **10µg/ml a-Human Insulin-like Growth Factor I** in PBS overnight at 4°C. Also run a negative control (no primary antibody) to check for non-specific staining.
6. Wash the sections with PBS four times, 15 minutes each, at room temperature.
7. Incubate the sections with a **1:100 dilution of goat anti-mouse** fluorescein conjugated secondary antibody in 1% BSA for 2 hours at room temperature.
8. Wash the section with PBS three times, 15 minutes each.
9. Examine the sections under a fluorescent microscope.

a-IGF-I Neutralization Assay Protocol

1. 2 days prior to the assay, seed cells in 2% serum to a density of 5×10^4 cells/ml, in a 24 well tissue culture plate.
2. 1 day prior to the assay, serum starve the cells.
3. On the day of the assay, pre-incubate IGF-I (1-20ng/ml) with different concentrations of **a-IGF-I** (0-30µg/ml) for 1 hour at 37°C in serum free media.
4. Aspirate the media off the cells and add the pre-incubated IGF-I/ α -IGF-I mixture in serum free media to the cell layer. Incubate for 24 hours at 37°C, 5% CO₂. Include appropriate positive and negative controls.
5. Aspirate off the media and pulse with 1µCi ³H-thymidine/0.5ml media, 2% FBS, for 4 hours at 37°C, 5% CO₂.
6. Aspirate off the media containing the ³H-thymidine into a radiation approved container, and wash each well twice, with 1ml ice-cold 5% TCA. Note: collect the washes into a radiation approved container.
7. Solubilize the cells using 0.8ml 0.5M NaOH/well. Mix and transfer 0.7ml to 5ml of scintillation fluid and count using a scintillation counter.