

Anti-IGF-I, clone Sm1.2

(mouse monoclonal IgG_{1κ})

Catalog # 05-172

Lot # 26433

Immunogen: Human insulin-like growth factor I (IGF-I) purified from human plasma.⁴

Specificity: Recognizes and is specific to IGF-I.³ Approximately 40% cross reactivity to IGF-II.⁵

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

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

Formulation: 200μg of protein G purified mouse IgG_{1κ}, lyophilized from 308μl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.1mM EDTA. Lyophilized powder.

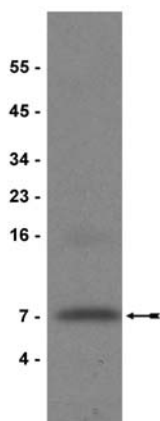
Storage and Stability: Lyophilized: Stable for 1 year at -20°C from date of shipment. Rehydrated: Rehydrate in 308μl sterile, distilled water; stable for 6 months at -20°C once rehydrated. Aliquot rehydrated solution to avoid repeated freezing and thawing.

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

Quality Control Testing and Research Applications

Immunoblot Analysis: 0.2-0.5μg/ml of this lot detected 100ng of IGF-I (Catalog # 01-208) under non-reducing conditions.

Immunoprecipitation: 5μg of previous lots immunoprecipitated 100ng IGF-I.



Immunoblot Analysis
IGF-I was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-IGF-I (0.5μg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemi-luminescence detection system. Arrow indicates IGF-I (7.6kDa).

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

Neutralization: 10-20μg/ml of this antibody can inhibit the activity of 10ng/ml of IGF-I as determined by testing with chicken embryo fibroblasts (CEF) using an ATP endpoint assay (ATPLite™-M, Packard Instruments).

Application References:

1. Harvey, A., *et al.*, *J. Biol. Chem.* **274**: 23249-23255, 1999.
2. Manes, S., *et al.*, *J. Biol. Chem.* **274**: 6935-6945, 1999.

References:

3. Russell, W.E., *et al.*, *Proc. Natl. Acad. Sci. USA.* **81**: 2389-2392, 1984.
4. van Wyk, J.J., *et al.*, *Human Growth Hormone* 585-599, Plenum Publishing Corp, NY 1986.
5. van Wyk, J.J., *et al.*, *Endocrinology* **138**: 4521, 1997.
6. Lui, *et al.*, *Mol. Cell. Neurosci.* **5**: 418-429, 1994.

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 (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.2-0.5µg/ml of anti-IGF-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 HRP conjugated IgG, Catalog # 12-349, 1:2000 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 1 µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **5µg of anti-IGF-I** to the sample.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl (50µl packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266).
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 60µl 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 anti-IGF-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 in the dark, 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.

Neutralization Assay

1. 2 days prior to the assay, seed cells in 2% serum to a density of 10⁵ cells/ml, in a 96 well, black 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 **anti-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/anti-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. Count wells using an ATP endpoint assay (ATPLite™-M, Packard Instruments). ATP endpoint assay was

performed following vendor's instructions. Cell layer was washed one time in PBS, prior to lysis.