

Anti-IGF-I, clone Sm1.2

Monoclonal Antibody

Cat. # 05-172

Lot # 27835

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP, IH, NEUT	Ch, H, M, R	IgG1κ	N/A	M	~17 kDa (migrates to ~7 kDa)	NP_000609

Background

Insulin-like growth factor 1 (IGF-1) is a polypeptide protein hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults. IGF-1 consists of 70 amino acids in a single chain with three intramolecular disulfide bridges.

IGF-1 is produced primarily by the liver as an endocrine hormone and targets tissues in a paracrine/autocrine fashion. Production is stimulated by growth hormone and can be retarded by undernutrition, growth hormone insensitivity, lack of growth hormone receptors, or failures of the downstream signaling pathway post GH receptor including SHP2 and STAT5b. Approximately 98% of IGF-1 is always bound to one of 6 binding proteins (IGF-BP). IGFBP-3, the most abundant protein, accounts for 80% of all IGF binding. IGF-1 binds to IGFBP-3 in a 1:1 molar ratio. Its primary action is mediated by binding to specific IGF receptors present on many cell types in many tissues. The signal is transduced by intracellular events. IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death.

Presentation

Purified mouse IgG1κ lyophilized powder, lyophilized from 105 µL of 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.1 mM EDTA.

Concentration

1 mg/mL after rehydration. See storage and handling.

Specificity

IGF-I. (Russel, W.E., 1984). 40% cross reactivity to IGF-II. (van Wyk, J.J., 1997).

Species Cross-reactivity

Human, mouse, rat and possibly chicken.

Occasional difficulty neutralizing rat IGF-I due to binding protein interference.

Immunogen

Human insulin-like growth factor I (IGF-I) purified from human plasma. (van Wyk, J.J., 1986).

Molecular Weight

17 kDa, migrates to ~7 kDa

Method of Purification

Protein G purified

Storage and Handling

Lyophilized: Stable for 1 year at -20°C from date of receipt.

Rehydrated: Rehydrate in 200 µL sterile, distilled water; stable for 6 months at -20°C once rehydrated. Aliquot rehydrated solution to avoid repeated freezing and thawing.

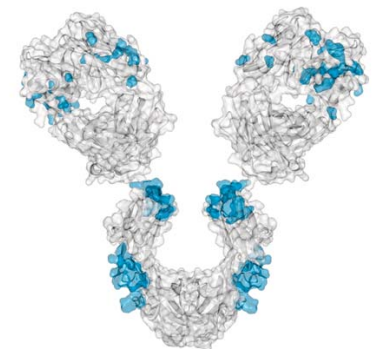
Control

MCF-7 cell extracts

Quality Control Testing

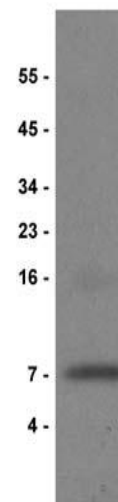
Evaluated on IGF-I (Catalog # 01-208) under non-reducing conditions.

Western Blot Analysis: 0.5-2 µg/mL of this antibody detected 100 ng of IGF-I (Catalog # 01-208) under non-reducing conditions.



References

1. Harvey, A. K., et al. (1999). *J Biol. Chem.* 274: 23249-55.
2. Manes, S., et al. (1999). *J Biol. Chem.* 274: 6935-45.
3. Russell, W.E., et al. (1984). *Proc. Natl. Acad. Sci. USA.* 81: 2389-2392.
4. van Wyk, J.J., et al. (1986). *Human Growth Hormone.* 585-599.
5. van Wyk, J.J., et al. (1997). *Endocrinology.* 138: 4521.
6. Lui, et al. (1994). *Mol. Cell. Neurosci.* 5: 418-429, 1994.



Western Blot Analysis:
Representative lot data. IGF-I was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-IGF-I (0.5 µg/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 kDa).

APPLICATION LEGEND: WB Western Blotting NEUT Neutralizing IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue)

SPECIES LEGEND: Ch Chicken H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research ApplicationsImmunoprecipitation:

5 µg of a previous lot immunoprecipitated
100 ng of IGF-I.

Immunohistochemistry:

10 µg/mL of a previous lot was reported by
an independent laboratory to detect IGF-I
in formalin-fixed, paraffin-embedded skin
sections.
(Lui, 1994).

Neutralization:

10-20 µg/mL of a previous lot inhibited the
activity of 10 ng/mL of IGF-I as determined by
testing with chicken embryo fibroblasts (CEF)
using an ATP endpoint assay (ATPLite™-M,
Packard Instruments).

PROTOCOL**Western Blotting**

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), (PBSMLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with 0.5-2 µg/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

1. Before beginning the immunoprecipitation, dilute the sample to roughly 1 µg/µL total cell protein in a microcentrifuge tube with PBS.
2. Add 5 µg of anti-IGF-I to the sample.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µL (50 µL 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 60 µL 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 10 µm 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 400 µL 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 10 µg/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 (1-20 ng/mL) with different concentrations of anti-IGF-I (0-30 µg/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.

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05-166	■ Anti-IGF-II, clone S1F2
05-656	■ Anti-IGF-IR, clone JBW902
04-298	■ Anti-IGF-1R (C-terminus), clone 7G11
05-694	■ Anti-Phospho-IGF-IR (Tyr1131)/InsR (Tyr1158), clone JY202
06-429	■ Anti-IGF-IR α subunit
CBL257	■ Anti-Insulin-like Growth Factor-1 Receptor, alpha-Subunit
CBL52	■ Anti-Insulin-like Growth Factor-I
MAB1120	■ Anti-Insulin-like Growth Factor-I Receptor, alpha-Subunit, clone 24-31
MAB1122	■ Anti-Insulin-like Growth Factor-I Receptor, alpha-Subunit, exon 7/8, clone 24-57
MAB1123	■ Anti-Insulin-like Growth Factor-I Receptor, beta-Subunit, clone 1-2
AB1438	■ Anti-Insulin-like Growth Factor-II
CBL82	■ Anti-Insulin-like Growth Factor-II, clone W2H1
AB1437	■ Anti-Insulin-like Growth Factor-I
AB2131P	■ Anti-Insulin-like Growth Factor-I
01-208	■ IGF-I
01-189	■ IGF-I (resistant to IGF-BPs)
01-212	■ IGF-I, biotin conjugate
01-142	■ IGF-II
12-131	■ IGF-BP-3
14-465	■ IGF-IR (Δ 1-958), active
12-527	■ IGF-IRtide
GF006	■ Insulin-like Growth Factor-I, recombinant human
GF121	■ Insulin-like Growth Factor-I, recombinant mouse
GF007	■ Insulin-like Growth Factor-II, recombinant human
S7842	■ CpG WIZ [®] H19-IGF2 Amplification Kit
32-070	■ IGF-IR KinEASE [™] FP Fluorescein Green Assay
32-150	■ IGF-IR KinEASE [™] FP-645nm FarRed Assay
M-003012	■ IGF-1R SMARTpool [®] siRNA reagent
62-115	■ siRNA plasmid, pKD-IGF-IR-v1
62-114	■ siRNA plasmid, pKD-IGF-IR-v2

RELATED PRODUCTS (non-specific)

cat #	description
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IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 μ m
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 μ m
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow [™] ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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