

**Anti-Human IGF-I Receptor, alpha-subunit
(chicken polyclonal IgY)**

Catalog # 06-429

Lot # 14493

Immunogen: 19 residue synthetic peptide [CKYADGTIDIEEV TENPKT] corresponding to amino acid residues (642-659) of human IGF-I receptor alpha-subunit.¹

Specificity: Specific to IGF-I receptor alpha-subunit.

Species Cross-reactivity: Recognizes human and mouse IGF-I receptor, does not recognize chicken IGF-I receptor, other species cross reactivity currently unknown.

Quantity and Formulation: 250µg of chicken IgY in 250µl PBS, pH 7.4, with 0.05% sodium azide. Purified via PEG and ammonium sulfate precipitation.

Physical Form: Frozen Liquid.

Storage and Stability: 1 year at -20°C.
Avoid repeated freezing and thawing.

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.

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

Quality Control Testing and Research Applications

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

Immunoprecipitation: 5 μ g of this lot immunoprecipitated the IGF-I receptor alpha subunit from membranes prepared from 3T3 cells expressing the human IGF-I receptor.

Western Blot Analysis: 1 μ g/ml of this lot detected the IGF-I receptor alpha subunit from a serum-starved DU145 prostate cell lysate and membranes prepared from 3T3 cells expressing the human IGF-I receptor. The specificity of the antibody binding was confirmed by blocking the reaction with the immunizing peptide (Upstate Biotechnology Catalog # 12-168)

Immunocytochemistry: 10 μ g/ml of this lot detected the IGF-I receptor on 2% paraformaldehyde/1% acetic acid-fixed 3T3 cells expressing the IGF-I receptor.

Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 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 μ g/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) 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 (MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **1ng/ml of α -Human IGF-I Receptor alpha-subunit**, 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 rabbit anti-chicken IgY linked to horseradish peroxidase, 1:1000 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. **Soak nitrocellulose in water for 1/2-1 hour prior to development to reduce background.**
10. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Before beginning the immunoprecipitation, dilute the cell lysate to approximately 1 μ g/ μ l total cell protein in a microcentrifuge tube with PBS.
2. Add **5ng of α -Human IGF-I Receptor** to 500 μ g-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l of immobilized rabbit anti-chicken IgY. **IgY does not effectively bind to either protein A or G.**
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 ice-cold PBS.
7. Resuspend the agarose beads in 50 μ l 2X Laemmli sample buffer.
8. Vortex beads and pulse spin to pellet the beads.
9. Remove the 2X Laemmli sample buffer and either freezer for later use or boil the supernatant for SDS-PAGE immunoblot analysis.

Immunocytochemistry

1. Plate approximately 200 μ l of cell suspension into each well of a slide. Incubate 24 hrs in a 37°C CO₂ incubator.
2. Add 200 μ l fix (ice-cold paraformaldehyde/acetic acid [4%/2%] in PBS) to 200 μ l media in each well for 15 minutes. This gives a final concentration of 2%/1%.
3. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
4. Add 400 μ l of 8% albumin in PBS and incubate for 30 minutes at room temperature.
5. Wash the cells with PBS, for 15 minutes.
6. Incubate the cells with 10 μ g/ml α -Human IGF-I Receptor alpha-subunit in 1% albumin in PBS and incubate overnight at 4°C.
7. Wash the cells twice with PBS, for 5 minutes.
8. Incubate the cells with a 1:100 dilution of rabbit anti-chicken IgY fluorescein conjugated secondary antibody in PBS for 1 hour at room temperature.
9. Wash the cells three times with PBS, for 5 minutes.
10. Examine the cells under a fluorescent microscope.