10 Old Barn Road · Lake Placid, NY 12946
Technical Support: T: 800 548-7853 · F: 518 523-4513
email: techserv@upstatebiotech.com
Sales Department: T: 800 233-3991 · F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstatebiotech.com

upstate® biotechnology
an Argonex Company

Certificate of Analysis

Anti-IGF-I Receptor a subunit

(chicken polyclonal IgY)
Catalog # 06-429
Lot # 21494

Immunogen: 19 residue synthetic peptide [CKYADGTIDIEEVTENPKT] 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.

Formulation: **250㎎** of chicken IgY in **250㎖** PBS, pH 7.4, with 0.05% sodium azide. Purified via PEG and ammonium sulfate precipitation. Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

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: 5µg of a previous lot immunoprecipitated the IGF-I receptor α subunit from membranes prepared from 3T3 cells expressing the human IGF-I receptor.

Western Immunoblot Analysis: 1-4µg/ml of this lot detected the IGF-I receptor α subunit from membranes prepared from 3T3 cells expressing the human IGF-I receptor. The specificity of the antibody binding has been confirmed by blocking the reaction with the immunizing peptide.

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

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.

Western Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell membrane preparation 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 **1-4µg/ml of anti-IGF-I Receptor α 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 HRP conjugated IgY, Catalog # 12-341, 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 membrane preparation to approximately 1µg/µl total cell protein in a microcentrifuge tube with PBS.
2. Add **5µg of ant-IGF-I Receptor α subunit** to 500µg-1mg membrane preparation.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100µl 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 50µl 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 200µl of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO_2 incubator.
2. Add 200µl fix (ice-cold paraformaldehyde/acetic acid [4%/2%] in PBS) to 200µl 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 400µl 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 **10µg/ml anti-IGF-I Receptor α-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.