



cell signaling solutions

Certificate of Analysis

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Anti-Clusterin α chain (human), clone 41D

(mouse monoclonal IgG_{1κ})

Catalog # 05-354

Lot # 24193

Immunogen: Native clusterin purified from human serum. Clone 41D.

Specificity: Recognizes human clusterin α chain, Mr 40kDa.

Species Cross-Reactivity: Human.

Formulation: 100 μ g of protein G purified mouse IgG_{1κ} in 100 μ l 0.1M Tris-glycine, pH 7.4, 0.15M NaCl and 0.05% sodium azide. Frozen solution.

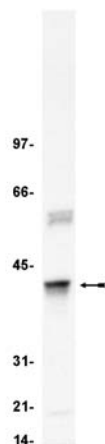
Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeating freezing and thawing. For maximum yield, centrifuge the original vial after thawing and prior to removing the cap.

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

Quality Control Testing and Research Applications

Immunoblot Analysis:^{1,2} 0.5-2 μ g/ml of this lot detected human clusterin α chain in RIPA lysates from HeLa cells; previous lots detected the protein in human serum and MCF-7 cell lysates.

Immunohistochemistry: Previous lots of this antibody at 5 μ g/ml detected clusterin in paraffin embedded human brain sections.



Immunoblot Analysis

Representative blot from a previous lot. HeLa cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Clusterin α chain (0.5 μ g/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates clusterin (~40kDa).

References:

1. Wilson, M.R., *et. al.*, Biochem. Biophys. Acta **1159**: 319-326, 1992.
2. Narvaez, C.J., *et. al.*, Endocrinology **137**: 400-409, 1996.

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 each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF), **or** a 1:10 dilution of human serum, 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 30-60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-Clusterin α chain (human)**, 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 (colorimetric detection was used).

Immunohistochemistry Protocol

1. Fix frozen brain sections with 4% paraformaldehyde/2% acetic acid in PBS for one minute at room temperature.
2. Wash the sections twice with PBS for 15 minutes, with very gentle agitation.
3. Cover the sections with 8% albumin in PBS and incubate for 30 minutes at room temperature.
4. Wash the sections with PBS, for 15 minutes, with very gentle agitation.
5. Incubate the sections overnight at 4°C with **5 μ g/ml of anti-Clusterin α chain (human)**, in PBS containing 1% albumin. Leave one section in 1% albumin in PBS as the negative control.
6. Wash the sections twice with PBS, for 30 minutes per wash.
7. Incubate the sections in the dark with a secondary reagent of choice (a goat anti-mouse IgG conjugated to fluorescein at a 1:100 dilution in PBS was used) for 30 minutes, in the dark.
8. Wash the sections three times with PBS, for 30 minutes per wash, in the dark.
9. Mount slides with gel mount, place cover slip, and allow gel to dry in the dark.
10. Examine sections with a fluorescent microscope and record photographically.