

## Certificate of Analysis

**Anti-Clusterin  $\alpha$  chain (human), clone 41D**  
(mouse monoclonal IgG<sub>1 $\kappa$</sub> )  
Catalog # 05-354  
Lot # 0609040503

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

**Specificity:** Recognizes human clusterin  $\alpha$  chain, Mr 40kDa, and precursor protein.

**Species Cross-Reactivity:** Human.

**Formulation:** 100  $\mu$ g of protein G purified mouse IgG<sub>1 $\kappa$</sub>  in 126 $\mu$ L of storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide). Frozen at -20°C.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

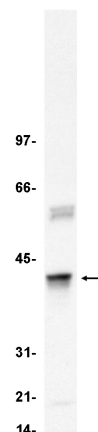
**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.**

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

### Quality Control Testing and Research Applications

**Immunoblot Analysis:**<sup>1,2</sup> 0.5-2  $\mu$ g/mL of this lot detected human clusterin  $\alpha$  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  $\mu$ 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  $\alpha$  chain (0.5  $\mu$ g/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates clusterin (~40 kDa).

#### Application References:

1. Caccamo, A. E., *et al* (2004). Cell detachment and apoptosis induction of immortalized human prostate epithelial cells are associated with early accumulation of a 45 kDa nuclear isoform of clusterin. *Biochem J* **382**: 157-68.
2. Scaltriti, M., *et al* (2004). Clusterin overexpression in both malignant and nonmalignant prostate epithelial cells induces cell cycle arrest and apoptosis. *Br J Cancer* **91**: 1842-50.

3. Narvaez, C J, *et al* (1996). Characterization of a vitamin D3-resistant MCF-7 cell line. *Endocrinology* **137**: 400-9.
4. Wilson, M R and Easterbrook-Smith, S B (1992). Clusterin binds by a multivalent mechanism to the Fc and Fab regions of IgG. *Biochim Biophys Acta* **1159**: 319-26.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM 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 µg/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, 1:5000 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 µg/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.