

# Anti-Clusterin $\alpha$ chain (human), clone 41D

Monoclonal Antibody

Cat. # 05-354

Lot # DAM1460168

pack size: 100  $\mu$ g

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS

Store at -20°C



## Certificate of Analysis

page 1 of 2

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IH(P)	H	IgG1k	N/A	M	40 kDa	NM_203339

### Background

Clusterin is also known as apolipoprotein J, complement lysis inhibitor, gp80, glycoprotein III, SGP-2, SP 40-40, TRPM2 and T64. It is a ubiquitous multifunctional protein of 80kD comprising of two disulfide-linked subunits, alpha and beta, and capable of interacting with a broad range of molecules. It is implicated in numerous biological processes including sperm maturation, lipid transport, regulation of the complement cascade, membrane recycling, cell death, immune regulation, cell adhesion and morphological transformation. In pathological conditions, it is an amyloid associated protein co-localising with fibrillar deposits in systemic and localised amyloid disorders. In Alzheimer's disease, clusterin is present in amyloid plaques and cerebrovascular deposits. In breast cancers, clusterin expression was found to correlate with tumour size. Clusterin is also implicated with endometrial cancers, myocardial infarction, renal diseases and atherosclerosis.

### Presentation

Purified mouse monoclonal IgG1k in buffer containing 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, with 0.05% sodium azide. Frozen at -20°C.

### Concentration

1 mg/mL

### Specificity

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

### Species Cross-reactivity

Human.

### Immunogen

Native clusterin purified from human serum. Clone 41D.

### Molecular Weight

40 kDa

### Method of Purification

Protein G Purified.

### Storage and Handling

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

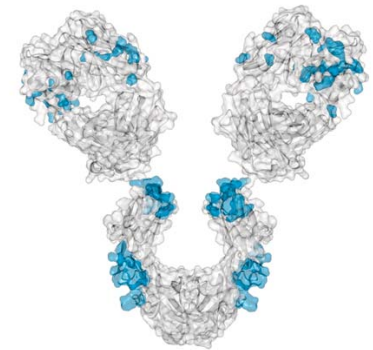
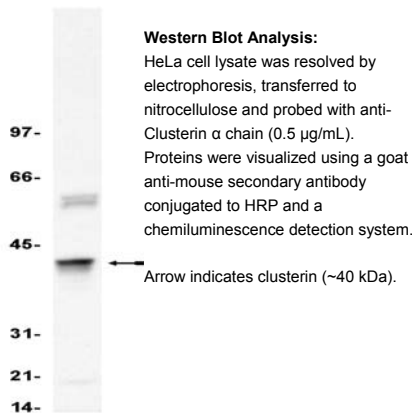
### Control

HeLa cell lysate.

### Quality Control Testing

Routinely evaluated on RIPA lysates from HeLa cells or MCF-7 cell lysates.

Western Blot Analysis: 0.5-2  $\mu$ g/mL of a previous 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



### References

1. Caccamo, A. E., et al. (2004). *Biochem J.* 382:157-68.
2. Scaltriti, M., et al. (2004). *Br J Cancer.* 91:1842-50.
3. Narvaez, C.J., et al. (1996). *Endocrinology.* 137:400-9.
4. Wilson, M.R. and Easterbrook-Smith, S.B., et al. (1992). *Biochim Biophys Acta.* 1159:319-26.

### Additional Research Applications

Immunohistochemistry: Previous lots of this antibody at 5  $\mu$ g/mL detected clusterin in paraffin embedded human brain sections.

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH(P) Immunohistochemistry (Paraffin)

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

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**PROTOCOL****Western Blot**

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  $\mu$ g/mL each aprotinin, leupeptin, pepstatin; 1 mM  $\text{Na}_3\text{VO}_4$ ; 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  $\mu$ g/mL of anti-Clusterin  $\alpha$  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**

1. Fix frozen brain sections with 4% paraformaldehyde and 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  $\mu$ g/mL of anti-Clusterin  $\alpha$  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.

**RELATED PRODUCTS (specific)**

cat #	description
05-356	■ Anti-Clusterin $\alpha$ chain (rat), clone 7A8-E4
12-349	■ Goat Anti-Mouse IgG, HRP conjugate

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 $\mu$ m
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 $\mu$ 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 $\mu$ 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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