

Product Information

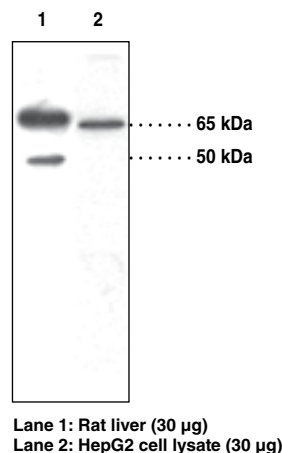


ACAT-1 Polyclonal Antibody

Item No. 100028 • Lot. No. XXXX

- Contents:** This vial contains (300 µg of peptide affinity-purified IgG, *lot specific*) lyophilized from 500 µl TBS, pH 7.4. Resuspend contents with 500 µl of water prior to use.
- Synonyms:** Acyl-Coenzyme A:Cholesterol Acyltransferase-1, Cholesterol Acyltransferase 1, SOAT1, Sterol O-Acyltransferase 1
- Antigen:** Human ACAT-1 amino acids 6-23; the antigen alignment with sequences from other species is as follows:
- | | |
|----------------------|--------------------------|
| Human | KMSLRNRLSKSRENPEED |
| Rat | e t SLRNRLS r S a ENPEqD |
| Mouse | m SLRNRLSKS g ENPEqD |
| Chinese hamster | KMSLRNRLSKS g ENPEqD |
| African green monkey | KMSLRNRLSKSRENPEED |
- Host:** Rabbit
- Cross Reactivity:** (+) Human, mouse, porcine, and rat ACAT-1; other species not yet tested
- Stability:** ≥1 year -20°C
- Applications:** The recommended starting dilution for western blotting is *lot specific: lot specific (lot specific* µg/ml) for a 1 hour incubation at room temperature. Overnight incubations at 4°C with greater dilutions can also produce optimal results.
- Concentration:** Varies by lot, from 0.2-1.0 mg/ml (100-500 µg/vial). Always 100 ml final working volume for western blotting.

Acyl-coenzyme A: cholesterol acyltransferase-1 (ACAT-1) catalyzes the formation of cholesterol esters from cholesterol and long chain fatty acyl-coenzyme A. ACAT-1 may be involved in maintaining appropriate membrane free cholesterol levels and in lipid droplet formation, and thus play a role in the development of atherosclerosis.^{1,2} ACAT-1 is ubiquitously expressed, with highest levels observed in sebaceous glands, steroidogenic tissues, and macrophages.³⁻⁵ This intracellular enzyme is located at the endoplasmic reticulum and has as many as eight transmembrane domains.¹ Human ACAT-1 has 550 amino acids with an estimated molecular weight of 65 kDa.⁵ Cayman's affinity purified antibody recognizes a 50 kDa band in most cultured cell lines as reported in the literature.^{4,6,7} In addition, the antibody reacts with a native form of ACAT-1 at about 65 kDa in liver tissues.



Laboratory Procedures

Immunofluorescent staining of cultured cells

1. Wash (attached) cells briefly with TBS and fix cells 10 minutes in 1% formalin in TBS, pH 7.4.
2. Wash cells 3 times in TBS, pH 7.4, 5 minutes each. For immunoperoxidase staining, follow steps 4-14 under the immunoperoxidase immunohistochemical procedure described below.
3. Incubate cells with 5% normal serum from the same species as the host of the secondary antibody in TBS, pH 7.4, containing 0.1% Triton X-100 (TBSTX) for 30 minutes.
4. Incubate cells with 0.4 µg/ml polyclonal antibody in TBSTX, pH 7.4, (recommended starting dilution; optimal dilution to be determined by end user) for 1 hour at room temperature.
5. Wash cells 3 times in TBSTX, pH 7.4, 5 minutes each.
6. Incubate cells for 1 hour with an anti-rabbit antibody fluorophore conjugate in TBSTX, pH 7.4, using a dilution as recommended by provider.
7. Wash cells 3 times in TBSTX, pH 7.4, 5 minutes each.
8. Counter-stain cells if desired.
9. The stained cells are now ready to be examined under a fluorescent microscope.

WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY; NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery.

Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence.

This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

Copyright Cayman Chemical Company, 12/19/2011

Cayman Chemical

Mailing address
1180 E. Ellsworth Road
Ann Arbor, MI
48108 USA

Phone
(800) 364-9897
(734) 971-3335

Fax
(734) 971-3640

E-Mail
custserv@caymanchem.com

Web
www.caymanchem.com

Immunoperoxidase immunohistochemical procedure

A. Paraffin sections

1. Deparaffinize sections 3 times with xylene or xylene substitute 5 minutes each.
2. Rehydrate sections with 100% ethanol 2 times, 5 minutes each, followed by 95%, 90%, 80%, 70%, and 50% ethanol, 5 minutes each.
3. Rinse sections in distilled water for 5 minutes.
4. Block endogenous peroxidase activity with 0.3% H₂O₂ in water (use methanol instead of water in case of strong endogenous peroxidase activity) for 15 minutes.
5. Wash sections 3 times in TBS, pH 7.4, 5 minutes each.
6. Incubate sections with 5% normal serum from the same species as the host of the secondary antibody for 30 minutes.
7. Incubate sections with 0.4 µg/ml polyclonal antibody (recommended starting dilution. Optimal dilution to be determined by end user) overnight at 4°C in a humid chamber.
8. Wash sections 3 times in TBS, pH 7.4, 5 minutes each.
9. Incubate sections for 30 minutes with biotinylated secondary antibody using a dilution as recommended by provider.
10. Wash sections 3 times in TBS, pH 7.4, 5 minutes each.
11. Incubate sections for 30 minutes with ABC reagent using a dilution as recommended by provider.
12. Wash sections 3 times in TBS, pH 7.4, 5 minutes each.
13. Incubate sections in peroxidase substrate solution. Check staining under a microscope frequently. When desired staining intensity is achieved, rinse sections with distilled water thoroughly.
14. Counter stain sections if desired. Rinse sections thoroughly after counter stain.
15. Dehydrate sections through 50%, 70%, 80%, 90%, 95%, and 100% (2 times) ethanol for 5 minutes each.
16. Clear sections with xylene or xylene substitute 3 times, 5 minutes.
17. Mount sections with coverslips.

B. Fresh frozen sections

1. After briefly fixing sections with an appropriate fixative (*e.g.*, 10% formaldehyde for 2 minutes), sections are washed with TBS, pH 7.4, 3 times, 5 minutes each.
2. Follow steps 4-18 of the procedure recommended for paraffin section.

References

1. Rudel, L.L., Lee, R.G., and Cockman, T.L. *Curr. Opin. Lipidol.* **12**, 121-127 (2001).
2. Linton, M.F. and Fazio, S. *Int. J. Obes.* **27**, S35-S40 (2003).
3. Namatame, I., Tomoda, H., Ishibashi, S., *et al. Proc. Natl. Acad. Sci. USA* **101**(3), 737-742 (2004).
4. Lee, R.G., Willingham, M.C., Davis, M.A., *et al. J. Lipid Res.* **41**, 1991-2001 (2000).
5. Chang, C.C.Y., Huh, H.Y., Cadigan, K.M., *et al. J. Biol. Chem.* **268**(28), 20747-20755 (1993).
6. Chang, C.C.Y., Lee, C.-Y.G., Chang, E.T., *et al. J. Biol. Chem.* **273**(52), 35132-35141 (1998).
7. Chang, C.C.Y., Chen, J., Thomas, M.A., *et al. J. Biol. Chem.* **270**(49), 29532-29540 (1995).

Related Products

For a list of related products please visit: www.caymanchem.com/catalog/100028

Cayman Chemical

Mailing address

1180 E. Ellsworth Road
Ann Arbor, MI
48108 USA

Phone

(800) 364-9897
(734) 971-3335

Fax

(734) 971-3640

E-Mail

custserv@caymanchem.com

Web

www.caymanchem.com