

# Product Information

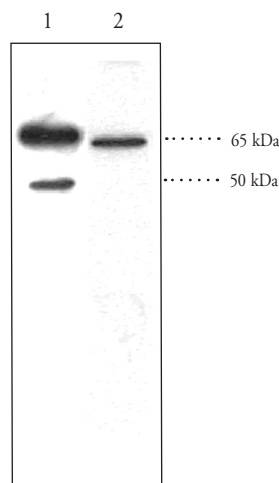


## ACAT-1 Polyclonal Antibody

Catalog No. 100028 • Lot. No. XXXX

- Synonyms:** Acyl-Coenzyme A: cholesterol acyltransferase-1; Cholesterol acyltransferase 1; Sterol O-acyltransferase 1
- Contents:** This vial contains XXX µg peptide affinity-purified IgG in XXX µl TBS, pH 7.4, containing 100 µg BSA and 0.02% sodium azide
- Host:** Rabbit
- 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 Sa ENPEqD |
| Mouse                | m SLRNRLSKS g ENPEqD    |
| Chinese hamster      | KMSLRNRLSKS g ENPEqD    |
| African green monkey | KMSLRNRLSKSRENPEED      |
- Cross-reactivity:** (+) Human, murine, porcine, and rat ACAT-1; other species not yet tested
- Stability:** ≥1 year -20°C
- Applications:** The recommended starting dilution for western blotting is X:XXX (X µg/ml) for a 1 hour incubation at room temperature. Overnight incubations at 4°C with greater dilutions can also produce optimal results. The recommended starting dilution for immunohistochemistry and immunocytochemistry is X:XXX (X µg/ml).

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.<sup>1,2</sup> ACAT-1 is ubiquitously expressed, with highest levels observed in sebaceous glands, steroidogenic tissues, and macrophages.<sup>3-5</sup> This intracellular enzyme is located at the endoplasmic reticulum and has as many as eight transmembrane domains.<sup>1</sup> Human ACAT-1 has 550 amino acids with an estimated molecular weight of 65 kDa.<sup>5</sup> Cayman's affinity purified antibody recognizes a 50 kDa band in most cultured cell lines as reported in the literature.<sup>4,6,7</sup> In addition, the antibody reacts with a native form of ACAT-1 at about 65 kDa in liver tissues.



1. Rat liver (30 µg)  
2. HepG2 cell lysate (30 µg)

### 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 NOT FOR HUMAN OR ANIMAL DISEASE DIAGNOSIS OR THERAPEUTIC DRUG 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 under separate cover to the MSDS supervisor at your institution.

#### WARRANTY AND LIMITATION OF REMEDY

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### 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% H<sub>2</sub>O<sub>2</sub> 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

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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).
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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).
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### Related Products

ACAT-2 Polyclonal Antibody - Cat. No. 100027 • ACAT-1 Blocking Peptide - Cat. No. 10005090 • ACAT-2 Blocking Peptide - Cat. No. 10005091 • Athero-PAK - Cat. No. 10005292

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