

# Product Information

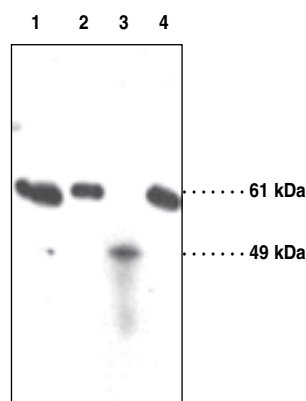


## ACAT-2 Polyclonal Antibody

Item No. 100027

- Synonyms:** Acyl-coenzyme A:Cholesterol Acyltransferase-2; Cholesterol Acyltransferase 2; Sterol O-Acyltransferase 2
- Contents:** This vial contains 250 µg peptide affinity-purified IgG in 500 µl TBS, pH 7.4, containing 50% glycerol, 0.1% BSA, and 0.02% sodium azide
- Host:** Rabbit
- Antigen:** Human ACAT-2 amino acids 3-20; the antigen alignment with sequences from other species is as follows:
- |                      |                                     |
|----------------------|-------------------------------------|
| Human                | PGGARLRLQRTEGLGGER                  |
| Rat                  | Pk a p q L R r r e r q G e e q E n  |
| Mouse                | P k v p q L R r r e g l G e e q E k |
| African green monkey | PGGARLRLQRTEGpGGER                  |
- Cross Reactivity:** (+) Human, mouse, rat, porcine, and ovine ACAT-2; other species not tested
- Storage:** ≥1 year at -20°C
- Applications:** The recommended starting dilution for western blotting is 1:250 (2 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 1:500 (1 µg/ml).

Acyl-coenzyme A:cholesterol acyltransferase-2 (ACAT-2) catalyzes the formation of cholesterol esters from cholesterol and long chain fatty acyl-coenzyme A. It is associated with lipoprotein particle secretion and thus plays an important role in the metabolism of triglyceride-rich lipoproteins like ApoB100.<sup>1-3</sup> In humans, ACAT-2 is primarily expressed in the small intestine, whereas in other mammals, the enzyme is expressed in the liver and the small intestine.<sup>2,4</sup> Human ACAT-2 has 522 amino acids with an estimated molecular weight of 60 kDa.<sup>5</sup> Cayman's antibody detects ACAT-2 at about 60 kDa in mouse liver, intestine, and fibroblasts.<sup>6</sup>



Lane 1: Murine liver (30 µg)  
Lane 2: Human fibroblast cell lysate (30 µg)  
Lane 3: Jurkat (human) cell lysate (30 µg)  
Lane 4: Rat liver (60 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 1 µg/ml polyclonal antibody (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 fluorochrome 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.

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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 1 µg/ml polyclonal antibody (recommended starting dilution; optimal dilution to be determined by end use) 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 3 times with xylene or xylene substitute 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 3 times with TBS, pH 7.4, 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. Acyl coenzyme A: cholesterol acyltransferase types 1 and 2: Structure and function in atherosclerosis. *Curr. Opin. Lipidol.* **12**, 121-127 (2001).
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6. Anderson, R.A., Joyce, C., Davis, M., *et al.* Identification of a form of acyl-CoA: cholesterol acyltransferase specific to liver and intestine in nonhuman primates. *J. Biol. Chem.* **273**(41), 26747-26754 (1998).

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