

# Affinity-purified Sheep Anti-human Apolipoprotein M/ApoM Antibody

## ORDERING INFORMATION

**Catalog Number:** AF4550

**Lot Number:** ZRB01

**Size:** 100 µg

**Formulation:** 0.2 µm filtered solution in PBS and 5% trehalose

**Storage:** -20° C

**Reconstitution:** sterile PBS and 0.02% NaN<sub>3</sub>

**Specificity:** human ApoM

**Immunogen:** *E. coli*-derived recombinant human ApoM (aa 23 - 188)

**Ig Type:** sheep IgG

**Application:** Western blot

## Background

Apolipoprotein M (ApoM; also known as Protein G3a) is a 25 kDa, secreted member of the lipocalin family of proteins. It is produced by hepatocytes and renal tubular epithelium, and represents a minor component of HDL particles. Human ApoM is 188 amino acids (aa) in length. It contains an uncleaved, hydrophobic N-terminal segment and one utilized N-linked glycosylation site (mouse ApoM shows no glycosylation). At least two potential isoforms exist. One shows an alternate start site at Met73, while a second shows the same start site plus a 21 aa substitution for aa 181 - 188. Human ApoM shares 91% and 80% aa identity with pig and mouse ApoM, respectively.

## Preparation

Sheep antibodies were raised against purified, *E. coli*-derived, recombinant human Apolipoprotein M (rhApoM; aa 23 - 188; Accession # O95445). Polyclonal antibody was affinity-purified on a column derivatized with recombinant human ApoM and further purified by isolating the IgG fraction.

## Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% NaN<sub>3</sub>. The antibody concentration will be 1.0 mg/mL.

## Storage

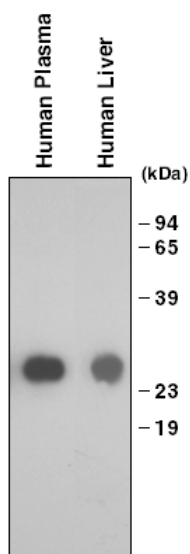
Lyophilized samples are stable for 12 months from date of receipt when stored at -20° C to -70° C. The reconstituted antibody should be aliquoted and stored at -20° C in a manual defrost freezer for 12 months without detectable loss of activity. **Avoid repeated freeze/thaw cycles.**

## Specificity

The antibody detects endogenous human ApoM in Western blots.

## Application

**Western blot** - An antibody concentration of 0.5 µg/mL is recommended.



### Detection of ApoM with AF4550.

Human plasma and human liver tissue in SDS-sample buffer was resolved by SDS-PAGE, transferred to an Immobilon-P membrane, and immunoblotted with 0.5 µg/mL sheep anti-ApoM as described in *Protocols for Immunoblotting*.

## Protocols for Immunoblotting

### Blotting Buffer

25 mM Tris, pH 7.4  
0.15 M NaCl  
0.1% Tween<sup>®</sup> 20

### Blocking Solution

5% nonfat dry milk  
in Blotting Buffer  
Adjust pH to 7.4

### Antibody Solution

2% nonfat dry milk  
in Blotting Buffer  
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8°C in Antibody Solution containing 0.5 µg/mL anti-human ApoM.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:1,000 dilution of HRP-conjugated Donkey anti-Sheep IgG (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent substrate detection system according to manufactures protocol.

**Cell lysates for Western blottings** - To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at  $2 \times 10^6$  -  $1 \times 10^7$  cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1X SDS sample buffer to the desired concentration.

**Optimal dilutions should be determined by each laboratory for each application.**

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

**R&D Systems, Inc.**  
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