PRODUCT INFORMATION

Atherosclerosis Product Pack

Item No. 10005292

Storage: -20°C
Stability: As supplied, 6 months from the QC date provided on the Certificate of Analysis, when stored properly

Description

The Cayman Atherosclerosis Product Pack contains our human SOAT-1/ACAT-1 polyclonal antibody and blocking peptide. Targeting human amino acids 6-23 of the sterol O-acyltransferase 1/acyl-coenzyme A:cholesterol acyl transferase-1, these reagents can be used for western blot analysis. Also included are several oxidized bioactive lipid species, including cholesteryl linoleate hydroperoxides,[1,2] POV-PC,[3] and PGPC.[4] The potent cholesteryl ester transfer protein (CETP) inhibitor JTT-705 is also included, at no charge.[5]

POV-PC, PGPC, and cholesteryl linoleate hydroperoxides are provided as solutions in ethanol, Dalcetrapib is supplied as a crystalline solid, SOAT-1/ACAT-1 polyclonal antibody is supplied as lyophilized IgG, and SOAT-1/ACAT-1 blocking peptide is supplied as a solution in aqueous buffer. Specifics of formulations are given on the individual inserts. Please see the chart below for the amount included and solubility information for the items in this kit.

Component	Amount	Solubility
POV-PC	1 mg	>10 mg/ml in PBS (pH 7.2)
PGPC	1 mg	>5 mg/ml in PBS (pH 7.2)
Cholesteryl Linoleate Hydroperoxides	100 µg	<20 µg/ml in PBS (pH 7.2)
Dalcetrapib	1 mg	>1 mg/ml in EtOH:PBS (pH 7.2) (1:1)
SOAT-1/ACAT-1 Polyclonal Antibody	1 ea	N/A
SOAT-1/ACAT-1 Blocking Peptide	1 ea	N/A

References

1. Brooks, C.J.W., Harland, W.A., Steel, G., *et al.* Lipids of human atheroma: Isolation of hydroxyoctadecadienoic acids from advanced aortal lesions. *Biochim. Biophys. Acta* **202**, 563-566 (1970).
2. Lenz, M.L., Hughes, H., Mitchell, J.R., *et al.* Lipid hydroperoxy and hydroxy derivatives in copper-catalyzed oxidation of low density lipoprotein. *J. Lipid Res.* **31**, 1043-1050 (1990).
3. Podrez, E.A., Batyreva, B., Shen, Z., *et al.* Identification of a novel family of oxidized phospholipids that serve as ligands for the macrophage scavenger receptor CD36. *J. Biol. Chem.* **277(41)**, 38503-38516 (2002).
4. Leitinger, N., Tyner, T.R., Oslund, L., *et al.* Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils. *Proc. Natl. Acad. Sci. USA* **96(21)**, 12010-12015 (1999).
5. Okamoto, H., Yonemori, F., Wakitani, K., *et al.* A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature* **406**, 203-206 (2000).

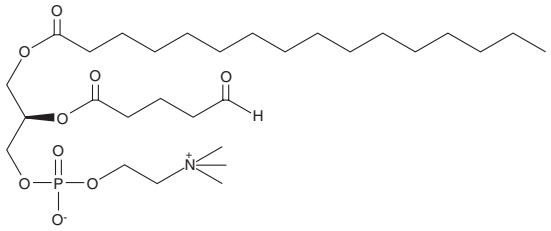
CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.CAYMANCHEM.COM

PRODUCT INFORMATION

POV-PC

Item No. 10031

CAS Registry No.: 121324-31-0
Formal Name: 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphatidylcholine
Synonym: 2-(5-oxovaleryl) Phosphatidylcholine
MF: $C_{29}H_{56}NO_9P$
FW: 593.7
Purity: ≥98%
Supplied as: A solution in ethanol
Storage: -20°C
Stability: As supplied, 1 year from the QC date provided on the Certificate of Analysis, when stored properly



Laboratory Procedures

POV-PC is supplied as a solution in ethanol. To change the solvent, simply evaporate the POV-PC under a gentle stream of nitrogen and immediately add the solvent of choice. Solvents such as ethanol, DMSO, and dimethyl formamide (DMF) purged with an inert gas can be used. The solubility of POV-PC in ethanol is approximately 30 mg/ml and approximately 10 mg/ml in DMSO and DMF.

Further dilutions of the stock solution into aqueous buffers or isotonic saline should be made prior to performing biological experiments. Ensure that the residual amount of organic solvent is insignificant, since organic solvents may have physiological effects at low concentrations. If an organic solvent-free solution of POV-PC is needed, it can be prepared by evaporating the ethanol and directly dissolving the neat oil in aqueous buffers. The solubility of POV-PC in PBS (pH 7.2) is approximately 10 mg/ml. We do not recommend storing the aqueous solution for more than one day.

Description

Oxidized low-density lipoprotien (oxLDL) particles contain low molecular weight species which are cytotoxic and pro-atherogenic.[1] Many of these substances were recently isolated and purified from oxLDL, and identified as phosphatidylcholine species containing a fragmented, oxidized short-chain fatty acid remnant at the *sn*-2 position.[2] 1-(Palmitoyl)-2-(5-oxovaleroyl)-phosphatidylcholine, or POV-PC, is one of the oxLDL species derived from 2-arachidonoyl or eicosapentanoyl phospholipids.[3] POV-PC confers CD36 scavenger receptor binding affinity more potently than any hydroperoxy PC species, and may be one of the more important structural determinants of oxLDL. Treatment of cultured endothelial cells with POV-PC stimulates monocyte binding, stimulates intracellular cAMP production, and strongly inhibits the LPS-induced binding of neutrophils.[4]

References

1. Podrez, E.A., Febbraio, M., Sheibani, N., *et al. J. Clin. Invest.* **105(8)**, 1095-1108 (2000).
2. Podrez, E.A., Batyreva, E., Shen, Z., *et al. J. Biol. Chem.* **277(41)**, 38517-38523 (2002).
3. Podrez, E.A., Batyreva, B., Shen, Z., *et al. J. Biol. Chem.* **277(41)**, 38503-38516 (2002).
4. Leitinger, N., Tyner, T.R., Oslund, L., *et al. Proc. Natl. Acad. Sci. USA* **96(21)**, 12010-12015 (1999).

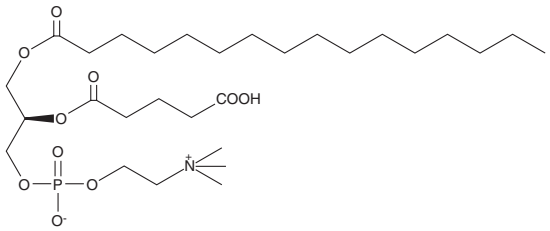
CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM

PRODUCT INFORMATION

PGPC

Item No. 10044

CAS Registry No.: 89947-79-5
Formal Name: 1-palmitoyl-2-glutaryl phosphatidylcholine
MF: $C_{29}H_{56}NO_{10}P$
FW: 609.7
Purity: ≥98%
Supplied as: A solution in ethanol
Storage: -20°C
Stability: As supplied, 1 year from the QC date provided on the Certificate of Analysis, when stored properly



Laboratory Procedures

PGPC is supplied as a solution in ethanol. To change the solvent, simply evaporate the ethanol under a gentle stream of nitrogen and immediately add the solvent of choice. Solvents such as ethanol and DMSO purged with an inert gas can be used. The solubility of PGPC in these solvents is approximately 20 and 1 mg/ml, respectively.

Further dilutions of the stock solution into aqueous buffers or isotonic saline should be made prior to performing biological experiments. Ensure that the residual amount of organic solvent is insignificant, since organic solvents may have physiological effects at low concentrations. If an organic solvent-free solution of PGPC is needed, it can be prepared by evaporating the ethanol and directly dissolving the neat oil in aqueous buffers. The solubility of PGPC in PBS (pH 7.2) is approximately 5 mg/ml. We do not recommend storing the aqueous solution for more than one day.

Description

Oxidized low-density lipoprotein (oxLDL) particles contain low molecular weight species which promote the differentiation of monocytes and activate polymorphonuclear leukocytes.[1] Many of these substances were recently isolated and purified from oxLDL, and identified as phosphatidylcholine species containing a fragmented, oxidized short-chain fatty acid remnant at the *sn*-2 position.[2] One of these substances isolated from oxLDL and identified as azelaoyl PAF is a potent PPARγ agonist.[3] 1-Palmitoyl-2-glutaryl phosphatidylcholine (PGPC) and 1-(palmitoyl)-2-(5-oxovaleroyl) phosphatidylcholine (POV-PC) are closely related compounds with strikingly different activity.[4] PGPC treatment of vascular endothelial cells induces the expression of both E-selectin and VCAM-1, and increases endothelieal cell binding by both neutrophils and monocytes. This contrasts with POV-PC treatment, which stimulates only monocyte binding, and strongly inhibits the LPS-induced binding of neutrophils.[4]

References

1. Tontonoz, P., Nagy, L., Alvarez, J.G.A., *et al. Cell* **93**, 241-252 (1998).
2. Podrez, E.A., Batyreva, E., Shen, Z., *et al. J. Biol. Chem.* **277(41)**, 38517-38523 (2002).
3. Davies, S.S., Pontsler, A.V., Marathe, G.K., *et al. J. Biol. Chem.* **276**, 16015-16023 (2001).
4. Leitinger, N., Tyner, T.R., Oslund, L., *et al. Proc. Natl. Acad. Sci. USA* **96(21)**, 12010-12015 (1999).

CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM

PRODUCT INFORMATION

Cholesteryl Linoleate Hydroperoxides

Item No. 48001

Formal Name: (±)-9-hydroperoxy-10E,12Z-octadecadienoic acid, cholesteryl ester; (±)-13-hydroperoxy-9Z,11E-octadecadienoic acid, cholesteryl ester

MF: $C_{45}H_{76}O_4$

FW: 681.1

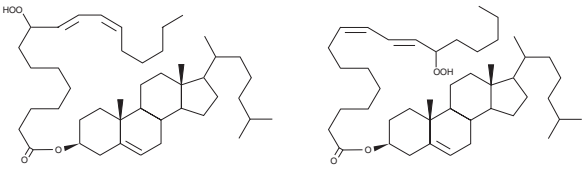
Purity: ≥98% hydroperoxide content

UV/Vis.: λ_{max}: 234 nm ε: 23,000

Supplied as: A solution in ethanol

Storage: -80°C

Stability: ≥6 months



Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Laboratory Procedures

Cholesteryl linoleate hydroperoxides are supplied as a solution in ethanol. To change the solvent, simply evaporate the ethanol under a gentle stream of nitrogen and immediately add the solvent of choice. A solvent such as dimethyl formamide (DMF) purged with an inert gas can be used. The solubility of cholesteryl linoleate hydroperoxides in DMF is approximately 50 mg/ml.

Cholesteryl linoleate hydroperoxides are sparingly soluble in aqueous buffers (<20 µg/ml in PBS pH 7.2), therefore further dilutions of the stock solution into aqueous buffers or isotonic saline should be made prior to performing biological experiments. Ensure that the residual amount of organic solvent is insignificant since organic solvents may have physiological effects at low concentrations. Linoleate hydroperoxides are extremely unstable in aqueous solutions. We recommend that the cholesteryl linoleate hydroperoxides diluted in aqueous solution be used as soon as possible, preferably within 15 minutes.

Description

Cholesteryl linoleate hydroperoxides are derived from the autoxidation of cholesteryl linoleate and contain a mixture of racemic 9- and 13-HpODE cholesteryl esters. Oxidative modification of LDL is suggested to play an important role in atherosclerosis. (±)9- and (±)13-HODE cholesteryl esters were originally extracted from atherosclerotic lesions and shown to be produced by Cu^{2+}-catalyzed oxidation of LDL.[1,2] Later studies determined that 15-lipoxygenase, from rabbit reticulocytes and activated human monocytes, oxygenates cholesteryl linoleate to both 9- and 13-hydroperoxy linoleate cholesteryl esters.[3,4] Cholesteryl ester hydroperoxides may be transferred from LDL to HDL, reduced to the corresponding hydroxides, and cleared *via* the liver.[5,6]

References

1. Brooks, C.J.W., Harland, W.A., Steel, G., *et al. Biochim. Biophys. Acta* **202**, 563-566 (1970).
2. Lenz, M.L., Hughes, H., Mitchell, J.R., *et al. J. Lipid Res.* **31**, 1043-1050 (1990).
3. Belkner, J., Wiesner, R., Kühn, H., *et al. FEBS Lett.* **279**, 110-114 (1991).
4. Folcik, V.A. and Cathcart, M.K. *J. Lipid Res.* **35**, 1570-1582 (1994).
5. Sattler, W. and Stocker, R. *Biochem. J.* **294**, 771-778 (1993).
6. Fluiter, K., Vietsch, H., Biessen, E.A.L., *et al. Biochem. J.* **319**, 471-476 (1996).

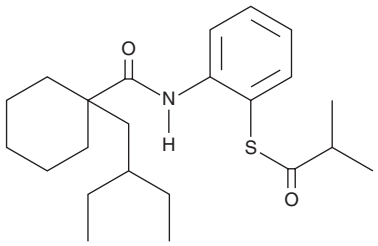
CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM

PRODUCT INFORMATION

Dalcetrapib

Item No. 89450

CAS Registry No.:	211513-37-0
Formal Name:	S-[2-[[[1-(2-ethylbutyl)cyclohexyl] carbonyl]amino]phenyl] propanethioic acid, 2-methyl ester
Synonym:	JTT-705
MF:	$C_{23}H_{35}NO_2S$
FW:	389.6
Purity:	≥98%
Supplied as:	A crystalline solid
Storage:	-20°C
Stability:	≥4 years



Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Laboratory Procedures

Dalcetrapib is supplied as a crystalline solid. A stock solution may be made by dissolving the dalcetrapib in an organic solvent purged with an inert gas. Dalcetrapib is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). The solubility of dalcetrapib in ethanol and DMF is approximately 30 mg/ml and appromixately 20 mg/ml in DMSO.

Dalcetrapib is sparingly soluble in aqueous buffers. For maximum solubility in aqueous buffers, dalcetrapib should first be dissolved in ethanol and then diluted with the aqueous buffer of choice. Dalcetrapib has a solubility of approximately 1 mg/mlin a 1:1 solution of ethanol:PBS (pH 7.2) using this method. We do not recommend storing the aqueous solution for more than one day.

Description

Cholesteryl ester transfer protein (CETP) is an exchange protein which transfers cholesteryl esters from HDL to VLDL, IDL, and LDL in exchange for triglycerides.[1,2] Dalcetrapib is an inhibitor of CETP that exhibits an IC_{50} value of 9 µM for plasma CETP in rabbits.[3] Inhibition of CETP by dalcetrapib in rabbits given an atherogenic diet leads to elevation of HDL, decreased VLDL, and attenuation of the induced atherosclerosis.[3] In human subjects, dalcetrapib also inhibits CETP activity and increases plasma HDL levels but it does not reduce the risk of recurrent cardiovascular events.[2,4]

References

1. Le Goff, W., Guerin, M., and Chapman, M.J. Pharmacological modulation of cholesteryl ester transfer protein, a new therapeutic target in atherogenic dyslipidemia. *Pharmacol. Ther.* **101**, 17-38 (2004).
2. Rader, D.J. Molecular regulation of HDL metabolism and function: Implications for novel therapies. *J. Clin. Invest.* **116(12)**, 3090-3100 (2006).
3. Okamoto, H., Yonemori, F., Wakitani, K., *et al.* A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature* **406**, 203-206 (2000).
4. Schwartz, G.G., Olsson, A.G., Abt, M., *et al.* Effects of dalcetrapib in patients with a recent acute coronary syndrome. *New England Journal of Medicine* **367(22)**, 2089-2099 (2012).

CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM

PRODUCT INFORMATION

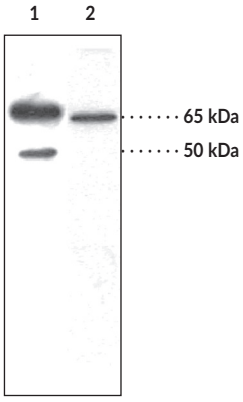
SOAT-1/ACAT-1 Polyclonal Antibody

Item No. 100028

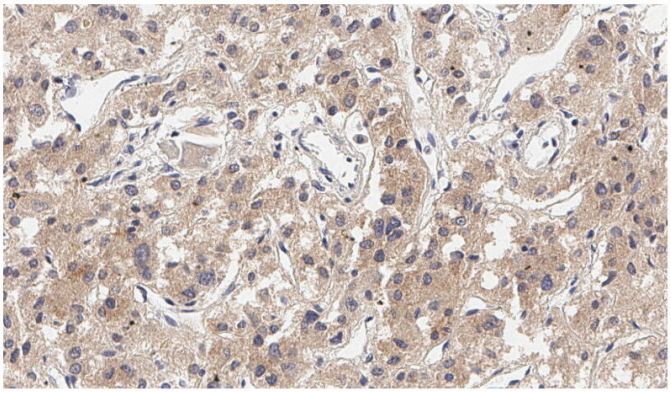
Overview and Properties

Contents:	This vial contains 500 µl of peptide affinity-purified polyclonal antibody.
Synonyms:	Acyl-coenzyme A: Cholesterol Acyltransferase-1, Cholesterol Acyltransferase 1, Sterol O-Acyltransferase 1
Immunogen:	Synthetic peptide from the N-terminal region of human SOAT-1/ACAT-1
Species Reactivity:	(+) Human, mouse, porcine, rat; other species not tested
Uniprot No.:	P35610
Form:	Liquid
Storage:	-20°C (as supplied)
Stability:	≥3 years
Storage Buffer:	PBS, pH 7.2, with 50% glycerol and 0.02% sodium azide
Host:	Rabbit
Application:	Immunohistochemistry (IHC) and Western blot (WB); the recommended starting dilution is 1:200. Other applications were not tested, therefore optimal working concentration/ dilution should be determined empirically.

Images



Lane 1: Rat liver (30 µg)
Lane 2: HepG2 cell lysate (30 µg)



Immunohistochemistry analysis of formalin-fixed, paraffin-embedded (FFPE) human adrenal tissue after heat-induced antigen retrieval in pH 6.0 citrate buffer. After incubation with SOAT-1/ACAT-1 Polyclonal Antibody, at a 1:200 dilution, slides were incubated with biotinylated secondary antibody, followed by alkaline phosphatase-streptavidin and chromogen (DAB).

CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM

PRODUCT INFORMATION

Description

Sterol O-acyltransferase 1 (SOAT-1), also known as acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT-1), is an enzyme encoded by *ACAT1* in humans that catalyzes the intracellular formation of cholesterol esters from cholesterol and long-chain fatty acyl-coenzyme A.[1] It is ubiquitously expressed and localized to the rough endoplasmic reticulum where it preferentially utilizes oleic acid (Item Nos. 90260 | 24659) or palmitic acid (Item No. 10006627) as fatty acid substrates for the synthesis of cholesterol esters, which are stored intracellularly or packaged into chylomicrons or VLDL and secreted into the blood stream. SOAT-1/ACAT-1 protein levels are increased in macrophages under various pathological conditions, including atherosclerosis.[2] SOAT-1/ACAT-1 activity is increased by cholesterol *in vitro*, and *ACAT1* expression is increased by stimulation with the pro-inflammatory cytokines TNF-α or IFN-γ in isolated human and THP-1 monocytes, respectively.[1,3] *ACAT1* silencing in human H4 neuroglioma cells overexpressing amyloid-β precursor protein (APP) reduces secretion of soluble amyloid-β (Aβ) and Aβ42 (Item No. 20574).[4] Genome-wide deletion of *Acat1* reduces macrophage infiltration and neutral lipid deposition in atherosclerotic aortic lesions and decreases serum total cholesterol levels, but increases brain cholesterol deposition, in *ApoE*$^{-/-}$ mice fed a Western diet.[5] Cayman's SOAT-1/ACAT-1 Polyclonal Antibody can be used for immunohistochemistry (IHC) and Western blot (WB) applications. The antibody recognizes the N-terminus to detect full-length SOAT-1/ACAT-1 at approximately 65 kDa from human, mouse, porcine, and rat samples.

References

1. Pramfalk, C., Eriksson, M., and Parini, P. Cholesteryl esters and ACAT. *Eur. J. Lipid Sci. Technol.* **114(6)**, 624-633 (2012).
2. Sakashita, N., Miyazaki, A., Chang, C.C.Y., *et al.* Acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT2) is induced in monocyte-derived macrophages: In vivo and in vitro studies. *Lab. Invest.* **83(11)**, 1569-1581 (2003).
3. Chang, T.-Y., Li, B.-L., Chang, C.Y., *et al.* Acyl-coenzyme A:cholesterol acyltransferases. *Am. J. Physiol. Endocrinol. Metab.* **297(1)**, E1-E9 (2009).
4. Huttunen, H.J., Greco, C., and Kovacs, D.M. Knockdown of ACAT-1 reduces amyloidogenic processing of APP. *FEBS Lett.* **581(8)**, 1688-1692 (2007).
5. Accad, M., Smith, S.J., Newland, D.L., *et al.* Massive xanthomatosis and altered composition of atherosclerotic lesions in hyperlipidemic mice lacking acyl CoA:cholesterol acyltransferase 1. *J. Clin. Invest.* **105(6)**, 711-719 (2000).

CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM

PRODUCT INFORMATION

SOAT-1/ACAT-1 Blocking Peptide

Item No. 10005090

Overview and Properties

Contents:	This vial contains 200 µg of peptide.
Synonyms:	Acyl-coenzyme A:Cholesterol Acyltransferase-1, Cholesterol Acyltransferase 1, Sterol O-Acyltransferase 1
Form:	Liquid
Storage:	-20°C (as supplied)
Storage Buffer:	200 µl TBS, pH 7.4, containing 0.1% BSA and 0.02% sodium azide
Stability:	As supplied, 1 year from the QC date provided on the Certificate of Analysis, when stored properly

Procedures

This vial contains 200 µg of peptide in 200 µl TBS, pH 7.4, containing 0.1% BSA and 0.02% sodium azide. This blocking peptide can be used in conjunction with Cayman's SOAT-1/ACAT-1 Polyclonal Antibody (Item No. 100028) to block protein-antibody complex formation during immunochemical analysis of SOAT-1/ACAT-1.

To block antibody/protein complex formation, the following procedure is recommended:

1. Mix the SOAT-1/ACAT-1 Polyclonal Antibody (Item No. 100028) and blocking peptide together in a 1:1 (v/v) ratio in a microfuge tube. For example, mix 20 µl of antibody and 20 µl of peptide.*
2. Incubate for one hour at room temperature with occasional mixing prior to further dilution and application of the mixture to the immunoblot.
3. Dilute the mixture to the final working antibody concentration and apply to the slide or membrane as usual.

*This is a recommended mixture. The minimum amount of peptide needed for complete blocking has not been precisely determined and may vary depending on the sample being analyzed. The amount of peptide required may need to be increased if sufficient blocking does not occur.

CAYMAN CHEMICAL
1180 EAST ELLSWORTH RD
ANN ARBOR, MI 48108 · USA
PHONE: [800] 364-9897
[734] 971-3335
FAX: [734] 971-3640
CUSTSERV@CAYMANCHEM.COM
WWW.**CAYMANCHEM**.COM