Recombinant Human ACAT1 His-tag

Catalog Number: 10242-AC

DESCRIPTION

Source	*E. coli*-derived human ACAT1 protein Val34-Leu427 Accession # P24752 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	41 kDa, under reducing conditions
Activity	Measured by its ability to convert acetoacetyl-CoA and CoA into acetyl-CoA. The specific activity is >15000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 25 mM $MgCl_2$, 50 mM KCl, 0.5 mM DTT, pH 8.0
- Recombinant Human Acetyl-CoA Acetyltransferase 1 (rhACAT1) (Catalog # 10242-AC)
- Coenzyme A sodium salt hydrate (CoA) (Sigma, Catalog # C3144), 50 mM stock in deionized water
- Acetoacetyl coenzyme A sodium salt hydrate (AACoA) (Cayman Chemical, Catalog # 21219), 40 mM stock in deionized water
- UV Plate (Costar, Catalog # 3635)
- Plate Reader (Model: SpectraMax M5 by Molecular Devices) or equivalent

Assay

1. Dilute rhACAT1 to 0.05 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 100 µM CoA and 30 µM AACoA in Assay Buffer.
3. Load 50 µL of 0.05 µg/mL rhACAT1 into a plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read in kinetic mode for 5 minutes at an absorbance of 303 nm.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol x (-1)}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using extinction coefficient 16900 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Final Assay Conditions

Per Well:

- rhACAT1: 0.0025 µg
- CoA: 50 µM
- AACoA: 15 µM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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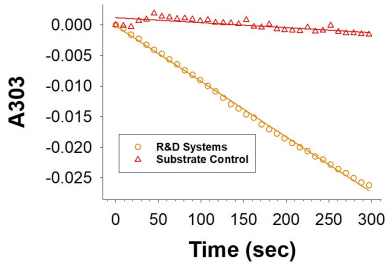
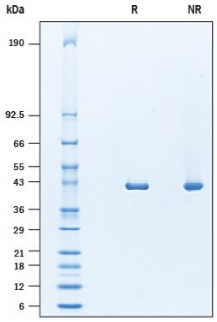
Enzyme Activity



Recombinant Human ACAT1 Protein His-tag Protein Enzyme Activity Recombinant Human ACAT1 His-tag (Catalog# 10242-AC) is measured by its ability to convert acetoacetyl-CoA and CoA into acetylCoA.

SDS-PAGE



Recombinant Human ACAT1 Protein His-tag Protein SDS-PAGE 2 µg/lane of Recombinant Human ACAT1 (Catalog # 10242-AC) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 41 kDa under reducing conditions.

BACKGROUND

Acetyl-CoA acetyltransferase-1 (ACAT1), also known as T2 or 3-ketothiolase, is a ubiquitous metabolic enzyme that catalyzes the potassium-regulated reversible thiolytic cleavage of fatty acids to form acetyl-CoA and a fatty acid acyl-CoA or condensation of two acetyl-CoA molecules into acetoacetyl-CoA (1). Human ACAT1 is a member of the thiolase superfamily and forms a homotetramer composed of a dimer of dimers due to an extended protruding loop (2). Each subunit contains an N-terminal domain including the dimer interface and a reactive cysteine while the C-terminal domain contains a catalytic pair that interacts with the N-terminal cysteine to create a catalytic triad (2). ACAT1 is known to be catalytically involved in isoleucine degradation, ketolysis, ketogenesis and fatty acid oxidation (3). Several mutations in the ACAT1 gene have been identified that lead to ACAT1 deficiency as an autosomal recessive inherited disorder known as 3-ketothiolase deficiency (3KTD) (4, 5). 3KTD is characterized by isoleucine degradation and defects in ketone body metabolism (6). More recently, ACAT1 has been shown to be involved in metabolic dysregulation in cancer through a role in drug resistance, cancer cell proliferation and tumor growth (3, 7-9). ACAT1 has been found to be upregulated as a potential prognostic marker in prostate cancer (3, 10) while overexpression of ACAT1 in breast cancer cells showed ACAT1's role in promoting tumor growth and metastasis through ketone body re-utilization (3, 11). ACAT1 inhibitors have been shown to inhibit proliferation of cancer stem cells (7, 12) possibly through inhibition of the ACAT1 acetyltransferase activity targeting pyruvate dehydrogenase and pyruvate dehydrogenase phosphatase (7) thus making ACAT1 a potential therapeutic target in cancer (3, 7, 11, 12).

References:

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