

Catalog Number: 10242-AC

Val34-Lev427 Accession # P24752 with an N-terminal Met and 6-His tag Harminal Sequence Met analysis redicted Molecular 42 kDa ass USP-PAGE 41 kDa, under reducing conditions totkivity Measured by its ability to convert acetoacely-ICOA and CoA into acetyI-COA. The specific activity is >1000 pmol/min/ug, as measured under the described conditions. indotoxin Level <0.10 EU per 1 ug of the protein by the LAL method. vurity >95%, by SDs-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining. formulation Supplied as 0.2 µm filtered solution in Tris, NaCl, Glycerol and TCEP. See Certificate of Analysis for details. tetivity Assay Protocol Recombinant Human Acetyl-CoA Acetyltransferase 1 (rhaCAT) (Catalog # 10242-AC) Coccuryme A sodium salt hydrate (CAA CoA) (Sigma, Catalog # 2014). 40 MM stock in deionized water Acetacetyl coenzyme A sodium salt hydrate (CAACA) (Cayanan Chemical, Catalog # 21219). 40 MM stock in deionized vater • Acetacetyl coenzyme A sodium salt hydrate (CAACA) (Cayanan Chemical, Catalog # 21219). 40 MM stock in deionized vater • Loed of Lo 0.05 µg/mL rhACAT1 into a ptate, and start the reaction by adding 50 µL of Substrate Mixture. Include containing 50 µL of 0.05 µg/mL rhACAT1 into a ptate, and start the		
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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: Specific Activity (pmol/min/μg) = Adjusted V _{max} * (OD/min) x well volume (L) x 10 ¹² pmol/mol x (-1) ext. coeff** (M ⁻¹ cm ⁻¹) 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.		2. Prepare Substrate Mixture containing 100 μM CoA and 30 μM AACoA in Assay Buffer.
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 CoA: 50 μM CoA: 55 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.		
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 6 months from date of receipt, -20 to -70 °C as supplied. 	,	
 3 months, -20 to -70 °C under sterile conditions after opening. 		

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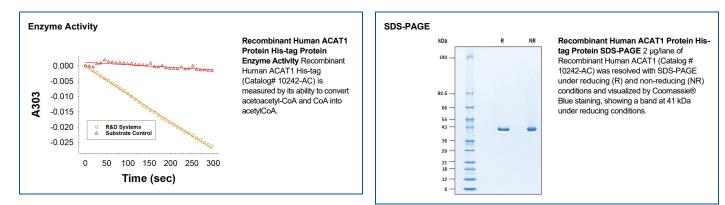


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RD SYSTEMS a biotechne brand

Recombinant Human ACAT1 His-tag

Catalog Number: 10242-AC



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 urpegulated 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 a epotential prognostic marker in prostate cancer (3, 7, 11, 12).

References:

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