Recombinant Mouse Carboxyl Ester Lipase/CEL

Catalog Number: 5658-CE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala21-Leu534, with a C-terminal 6-His tag Accession # Q64285
N-terminal Sequence Analysis	Ala21
Structure / Form	Monomer
Predicted Molecular Mass	58 kDa

SPECIFICATIONS

SDS-PAGE	60 kDa, reducing conditions
Activity	Measured by its ability to cleave p-Nitrophenyl butyrate (PNPB). The specific activity is >4,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, pH 7.0
- Reading Buffer: 25 mM Tris, pH 8.0
- Recombinant Mouse Carboxyl Ester Lipase/CEL (rmCEL) (Catalog # 5658-CE)
- Substrate: p-Nitrophenyl butyrate (PNPB) (Sigma, Catalog # N9876), 100 mM stock in acetone
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rmCEL to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Load into the wells of a clear microplate 50 µL of 0.2 ng/µL rmCEL, and start the reaction by adding 50 µL of Substrate. Include a Blank Control containing 50 µL of Assay Buffer and 50 µL of Substrate without any rmCEL.
4. Incubate 10 minutes at room temperature.
5. Quickly add 100 µL of Reading Buffer to all wells (this will not stop the reaction).
6. Read immediately at a wavelength of 410 nm (bottom read) in endpoint mode.
7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmol/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Nitrophenol (Sigma, Catalog # 241326).

Final Assay Conditions

Per Well:

- rmCEL: 0.010 µg
- Substrate: 0.5 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Carboxyl ester lipase, also known as bile salt-stimulated lipase, belongs to the Type-B carboxyl esterase/lipase family of enzymes. The enzyme possesses a Ser-His-Asp catalytic triad, and is capable of hydrolyzing both ester and amide bonds. CEL is a non-specific lipase which hydrolyzes a variety of substrates, including cholesteryl esters, acylglycerols, and ceramide (1). CEL is highly expressed by pancreatic acinar cells and secreted into the gastrointestinal tract, where it contributes to the digestion of dietary lipids (2). It is expressed at much lower levels by the liver, macrophages, and endothelial cells (3). CEL is present in the circulation, where it may play a role in low density lipoprotein metabolism and atherogenesis (4).

References:

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3. Hui, D.Y. and Howles, P.N. (2002) J. Lipid Res. **43**:2017.
4. Brodt-Eppley, J. *et al.* (1995) Biochim. Biophys. Acta. **1272**:69.

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