Recombinant Mouse Carboxylesterase 3/CES3

Catalog Number: 5428-CE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Tyr19-Glu561, with a C-terminal 10-His tag Accession # Q8VCT4
N-terminal Sequence Analysis	Tyr19
Predicted Molecular Mass	61 kDa

SPECIFICATIONS

SDS-PAGE	58 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze p-nitrophenylacetate. The specific activity is >11,000 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	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Mouse Carboxylesterase 3/CES3 (rmCES3) (Catalog # 5428-CE)
- Substrate: 4-Nitrophenyl acetate (4-NPA) (Sigma, Catalog # N-8130), 100 mM stock in Acetone
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rmCES3 to 0.2 ng/µL in assay buffer.
2. Dilute substrate 4-NPA to 2 mM in deionized water.
3. Load in a clear microplate 50 µL of 0.2 ng/µL rmCES3, and start the reaction by adding 50 µL of 2 mM 4-NPA. Include a blank consisting of 50 µL of assay buffer and 50 µL 2 mM 4-NPA.
4. Read at a wavelength of 400 nm (bottom read) in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rmCES3: 0.010 µg
- Substrate: 1 mM

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.

BACKGROUND

Carboxylesterase 3 is a member of a large family of carboxylesterases that are responsible for the hydrolysis of ester and amide bonds (1, 2). These carboxylesterases are widely distributed in mammalian tissues and have broad substrate specificity ranging from small molecule esters such as phenylester to long-chain fatty acid esters and thioesters. Because many ester-containing drugs require the ester linkage to improve the bioavailability of therapeutic agents, CESs play a major role in drug metabolism and activation. CES3 is also known as triacylglycerol hydrolase for its lipolytic activity. It has been reported to be the major lipase in white adipose tissue (3).

References:

1. Redinbo, M. R. and Potter, P.M. (2005) Drug Discovery Today. **10**:313.
2. Satoh, T. and Hosokawa, M. (2006) Chem.-Biol. Interactions. **162**:195.
3. Soni, K. G. *et al.* (2004) J. Biol. Chem. **279**:40683.

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