Recombinant Mouse Carboxylesterase 2/CES2

Catalog Number: 5280-CE

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Gln27-Lys557, with a C-terminal 10-His tag Accession # Q91WG0
N-terminal Sequence Analysis	No results obtained: Gln27 predicted
Predicted Molecular Mass	60 kDa

SPECIFICATIONS

SDS-PAGE	58 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze p-nitrophenylacetate. The specific activity is >35,000 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 Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, pH 7.5
- Recombinant Mouse Carboxylesterase 2/CES2 (rmCES2) (Catalog # 5280-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 rmCES2 to 0.4 ng/µL in Assay Buffer.
2. Dilute Substrate to 2 mM in deionized water.
3. In a plate load 50 µL of 0.4 ng/µL rmCES2, and start the reaction by adding 50 µL of 2 mM Substrate.
4. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of 2 mM Substrate.
5. Read at a wavelength of 400 nm (bottom read) in kinetic mode for 5 minutes.
6. 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:

- rmCES2: 0.02 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 2 is a member of a large family of carboxylesterases that are responsible for the hydrolysis of ester and amide bonds (1, 2). They have broad substrate specificity ranging from small molecule esters such as phenylester to long-chain fatty acid esters and thioesters. Carboxylesterases play a major role as determinants of pharmacokinetic behavior for most therapeutic agents containing an ester, participating in the detoxification of drugs such as cocaine and heroin in serum and liver. They can also detoxify organophosphate and carbamate analogues used in agrochemicals or chemical nerve agents, such as malathion, sarin, tabun, and VX. Carboxylesterases can also perform transesterification, a reaction important for cholesterol homeostasis. Carboxylesterase deficiency may be associated with non-Hodgkin lymphoma or B-cell lymphocytic leukemia. CES2, also known as acylcarnitine hydrolase M1, shares the serine hydrolase fold observed in other esterases (3). CES2 possesses an endoplasmic reticulum retention signal (HREL) at its C-terminus. The expressed recombinant mouse CES2 lacks this signal, resulting in its secretion.

References:

1. Redinbo, M. R. and P.M. Potter (2005) Drug Discovery Today **10**:313.
2. Satoh, T. and M. Hosokawa (2006) Chem.-Biol. Interactions **162**:195.
3. Fleming, C. D. *et al.* (2007) Biochemistry **46**:5603.

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