Recombinant Human Butyrylcholinesterase/BCHE

Catalog Number: 6137-CE

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Butyrylcholinesterase/BCHE protein Met1-Leu602, with a C-terminal 6-His tag Accession # P06276
N-terminal Sequence Analysis	Glu29
Structure / Form	Monomer and disulfide-linked homodimers
Predicted Molecular Mass	66 kDa

SPECIFICATIONS

SDS-PAGE	90-100 kDa, reducing conditions
Activity	Measured by its ability to cleave Butyrylthiocholine. The specific activity is >50,000 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 visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
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: 100 mM Sodium Phosphate, pH 7.5
- Recombinant Human Butyrylcholinesterase/BCHE (rhBCHE) (Catalog # 6137-CE)
- Substrate: Butyrylthiocholine chloride (BTC) (Sigma Catalog # B3128), 20 mM stock in DMSO
- 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) (Sigma Catalog # D8130), 10 mM stock in DMSO
- 96 well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhBCHE to 0.02 µg/mL in Assay Buffer.
2. Dilute BTC and DTNB to 200 µM final concentrations in deionized water.
3. Load into plate 50 µL of 0.02 µg/mL rhBCHE and start the reaction by adding 50 µL of the BTC/DTNB mixture to the wells. As a Substrate Blank, load 50 µL of Assay Buffer and 50 µL of the BTC/DTNB mixture.
4. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Using the extinction coefficient 13260 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhBCHE: 0.001 µg
- DTNB and BTC: 100 µM

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.

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BACKGROUND

Butyrylcholinesterase (BCHE) is a major acetylcholine hydrolyzing enzyme in the circulation (1). Although it is present in significant amounts in human plasma, no endogenous physiological substrate has been described for this enzyme. It can degrade a large number of ester-containing compounds besides acylcholines, including neurotoxic organophosphate esters. Thus, it plays significant pharmacological and toxicological roles. It is thought to be involved in the pathological progression of Alzheimer's disease (AD) by depleting acetylcholine. In contrast to ACHE, it attenuates amyloid fibril formation *in vitro* (2). BCHE inhibitors have been used to delay symptoms of AD patients by virtue of their ability to enhance ACH availability (3). Its involvement in the cholinergic anti-inflammatory pathway connects BCHE and ACHE as possible markers of low-grade systemic inflammation observed in Type-2 diabetes, obesity, hypertension, coronary heart disease, and AD (4). BCHE can exist as monomers, dimers, or tetramers (1).

References:

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3. Campbell, V. A. and Gowran, A. (2007) Br. J. Pharm. **152**:655.
4. Das, U. N. (2007) Med Sci Monit. **13**:RA214.

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