Recombinant Mouse Butyrylcholinesterase/BCHE

Catalog Number: 5527-CE

Background

Butyrylcholinesterase (BCHE) is a major acetylcholine hydrolyzing enzyme in the circulation (1). Although it is present in significant amounts (~ 3 mg/L) in human plasma, no endogenous physiological substrate has been described for this enzyme. It can degrade a large number of ester-containing compounds in addition to acylcholines. Thus, it is likely to play significant pharmacological and toxicological roles. It is thought to be involved in the pathological process 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 acetylcholine availability (3). Its involvement in a cholinergic anti-inflammatory pathway connect BCHE and ACHE with a possible marker of low-grade systemic inflammation observed in Type-2 diabetes, obesity, hypertension, coronary heart disease, and AD (4). BCHE can exist in monomeric and multimeric forms (1). The expressed recombinant mouse BCHE contains multiple forms that consist of soluble monomers, dimers, and tetramers.

References:

1. Darvesh, S. *et al*. (2003) Nat. Rev. Neuroscience **4**:131.
2. Diamant, S. *et al*. (2006) Proc. Natl. Acad. Sci. USA **103**:8628.
3. Campbell, V. A. and Gowran, A. (2007) Br. J. Pharm. **152**:655.
4. Das, U. N. (2007) Med Sci Monit. **13**:RA214.

Description

Source	Murine myeloma cell line, NS0-derived His28 - Leu603, with a C-terminal 6-His tag Accession # AAH99977
N-terminal Sequence Analysis	His28
Predicted Molecular Mass	66 kDa

Specifications

SDS-PAGE	86 kDa, reducing conditions
Activity	Measured by its ability to cleave Butyrylthiocholine. The specific activity is > 75 nmol/min/µg, as measured under the described conditions. See Activity Assay Protocol.
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.

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.

Activity Assay Protocol

Materials

- Assay Buffer: 100 mM Sodium Phosphate, pH 7.5
- Recombinant mouse BCHE (R&D Systems, Catalog # 5527-CE)
- Substrate: Butyrylthiocholine chloride (BTC) (Sigma Catalog #), 20 mM stock in DMSO
- 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) (Sigma Catalog # D-8130), 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 rmBCHE to 0.01 µg/mL in Assay Buffer.
2. Dilute BTC to 10 mM in deionized water.
3. Combine equal amounts of diluted BTC and DTNB. Dilute substrate mixture to a final concentration 200 µM BTC and DTNB in deionized water.
4. Load into plate 50 µL of 0.01 µg/mL rmBCHE 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.
5. Read in kinetic mode for 5 minutes at an absorbance of 405 nm.
6. Calculate specific activity:

$$\text{Specific Activity (nmoles/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^9 \text{ nmol/M}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*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

- rmBCHE: 0.0005 µg
- DTNB and BTC: 100 µM

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NOT FOR USE IN HUMANS.