Recombinant Human Acetylcholinesterase/ACHE
Catalog Number: 7574-CE

DESCRIPTION

Source
Chinese Hamster Ovary cell line, CHO-derived
Glu32-Leu614, with a C-terminal 6-His tag
Accession # P22303

N-terminal Sequence
Glu32

Analysis
Predicted Molecular Mass
65 kDa

SPECIFICATIONS

SDS-PAGE
60-75 kDa, reducing conditions

Activity
Measured by its ability to cleave Acetylthiocholine. The specific activity is >500 nmol/min/μg, as measured under the described conditions.

Endotoxin Level
<0.10 EU per 1 μg of the protein by the LAL method.

Purity
>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

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: 0.1 M Sodium Phosphate, 0.05% (w/v) Brij-35, pH 7.5
- Recombinant Human Acetylcholinesterase/ACHE (rhACHE) (Catalog # 7574-CE)
- Substrate: Acetylthiocholine (ATC) (Sigma, Catalog # A5626), 20 mM stock in DMSO
- 5',5'-Dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma, Catalog # D-8130), 10 mM stock in DMSO
- 96-well Clear Plate (Costar, Catalog # 92992)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay
1. Dilute rhACHE to 0.002 μg/mL in Assay Buffer.
2. Dilute Substrate to 200 μM in Assay Buffer containing 100 μM DTNB.
3. Load 50 μL dilute rhACHE to clear plate. Include a control containing Assay Buffer instead of rhACHE.
4. Start reaction by adding 50 μL Substrate/DTNB mix.
5. Read plate in kinetic mode for 5 minutes at an absorbance of 405 nm.
6. Calculate specific activity:

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

   *Adjusted for Substrate Blank
   **Using the extinction coefficient 13260 M^-1 cm^-1
   ***Using the path correction 0.32 cm

Final Assay Conditions
Per Well:
- rhACHE: 0.0001 μg
- DTNB: 50 μM
- ATC: 100 μM

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

The classical role of ACHE is to terminate cholinergic neurotransmission by hydrolysis of acetylcholine (ACH) (1). ACHE is thought to be involved in the pathology of Alzheimer’s disease (AD) by accelerating the assembly of Aβ peptides into fibrillar species through forming complexes with Aβ via the peripheral anionic site on ACHE. ACHE inhibitors have been used to delay symptoms of AD patients by virtue of their ability to enhance ACH availability, as well as reduce amyloidogenesis and subsequent neurotoxicity (2). Its involvement in the cholinergic anti-inflammatory pathway connects ACHE with a possible marker of low-grade systemic inflammation in obesity, hypertension, coronary heart disease, and AD (3). Alternative splicing produces three isoforms: an amphipathic form that exists as both monomeric and multimeric forms, a soluble monomeric form lacking the cysteine residue near the C-terminus, and a GPI-anchored dimeric form found in the membranes of erythrocytes (1). The recombinant human ACHE (rhACHE) was expressed as the amphipathic form that consists of soluble monomer and multimeric forms.

References:

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