Recombinant Human BACE-1

Catalog Number: 931-AS

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Thr22-Tyr460 (pro) & Glu46-Tyr460 (mature), both with a C-terminal 10-His tag Accession # P56817.1
N-terminal Sequence Analysis	Thr22 & Glu46
Structure / Form	Pro and Mature forms
Predicted Molecular Mass	50 kDa (Pro form) & 47 kDa (Mature form)

SPECIFICATIONS

SDS-PAGE	68-70 kDa and 65-67 kDa, reducing conditions
Activity	Measured by its ability to cleave a fluorogenic peptide substrate, Mca-SEVNLDAEFRK(Dpn)RR-NH_2 (Catalog # ES004). The specific activity is >3.5 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M Sodium Acetate, pH 4.0
- Recombinant Human BACE -1 (rhBACE-1) (Catalog # 931-AS)
- Fluorogenic Peptide Substrate IV: MCA-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(DPN)-Arg-Arg-NH_2 (Catalog # ES004)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhBACE-1 to 20 ng/µL in Assay Buffer.
2. Dilute Substrate to 20 µM in Assay Buffer.
3. Load 50 µL of rhBACE-1 into a plate and start the reaction by adding 50 µL of 20 µM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 20 µM Substrate.
4. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH (Bachem, Catalog # M-1975).

Final Assay Conditions

Per Well:

- rhBACE-1: 1 µg
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/mL in sterile, deionized water.
Shipping	The product is shipped at ambient temperature. 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 reconstitution.

BACKGROUND

BACE-1 is an aspartic protease and an integral membrane protein (1-5). BACE-1 is the peptidase predominantly responsible for cleavage of the amyloid precursor protein β site in the brain to generate the amyloid β peptide. Because the amyloid β peptide is a major component of amyloid plaques, BACE-1 has been implicated in the onset and/or progression of Alzheimer's disease. BACE-1 is expressed in a variety of human tissues. It is likely that this peptidase has functions in addition to its hydrolysis of the amyloid precursor protein. The peptidase activity of BACE-1 is optimal under mildly acidic conditions (pH 3.5-5.5), consistent with its proposed function in an acidic intracellular compartment.

References:

1. Ermolieff, J. *et al.* (2000) Biochemistry **39**:12450.
2. Lin, X. *et al.* (2000) Proc. Natl. Acad. Sci. USA **97**:1456.
3. Sinha, S. *et al.* (1999) Nature **402**:537.
4. Vassar, R. *et al.* (1999) Science **286**:735.
5. Yan, R. *et al.* (1999) Nature **402**:533.

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