R&D SYSTEMS
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Recombinant Mouse BACE-1

Catalog Number: 2976-AS

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Thr22-Thr457, with a C-terminal 10-His tag Accession # P56818
N-terminal Sequence Analysis	Thr22
Predicted Molecular Mass	50 kDa

SPECIFICATIONS

SDS-PAGE	68 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 >2 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	Lyophilized from a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Acetate, 100 mM NaCl, pH 4.0
- Recombinant Mouse BACE-1 (rmBACE-1) (Catalog # 2976-AS)
- Substrate: MCA-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(DNP)-Arg-Arg-NH_2 (Catalog # ES004)
- Heparin (Sigma, Catalog # H3393), 20 mg/mL in deionized water
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rmBACE-1 to 20 ng/µL in Assay Buffer.
2. Dilute Heparin to 4 ng/µL in Assay Buffer.
3. Combine equal volumes diluted Heparin and rmBACE-1.
4. Incubate at 37 °C for 30 minutes.
5. Dilute Substrate to 20 µM in Assay Buffer.
6. Load 50 µL of the rmBACE-1/heparin mixture in 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.
7. Read at excitation and emission wavelengths of 320 nm and 405 nm (top read), respectively, in kinetic mode for 5 minutes.
8. 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:

- rmBACE-1: 0.500 µg
- Substrate: 10 µM
- Heparin: 1 ng/µL

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 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.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

BACE-1 is an aspartic protease and an integral membrane protein (1, 2). It is the major β secretase, and together with the γ secretase, is responsible for generating the amyloid β peptide (Aβ) from the amyloid precursor protein (APP) (3, 4). Because Aβ is a major component of amyloid plaques, BACE-1 has been implicated in the onset and/or progression of Alzheimer's disease. High levels of BACE-1 activity are sufficient to elicit neurodegeneration and neurological decline in *vivo*, indicating that inhibiting BACE-1 may block not only Aβ-dependent but also Aβ-independent pathogenic mechanisms (5). In addition to APP, BACE-1 also cleaves APP-like proteins 1 and 2, the cell adhesion protein P-selectin glycoprotein ligand-1 and β-galactoside α2,6-sialyltransferase, implying that BACE-1 may have additional functions involving the ectodomain shedding of membrane proteins (6-8). The purified recombinant mouse BACE-1 corresponds to the ectodomain with the activity as described in Activity Assay Protocol.

References:

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