

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived human BACE-2 protein
Phe29-Pro466, with a C-terminal 10-His tag
Accession # Q9Y5Z0-1

N-terminal Sequence Analysis Phe29 & Ala63

Predicted Molecular Mass 49 kDa

SPECIFICATIONS

SDS-PAGE 48-60 kDa, reducing conditions

Activity Measured by its ability to cleave a fluorogenic peptide substrate Mca-KPLGL-Dpa-AR-NH₂ (Catalog # ES010).
The specific activity is >70 pmol/min/μg, as measured under the described conditions.

Endotoxin Level <0.10 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: 50 mM Sodium Acetate, 1 M NaCl, 0.05% Brij-35, pH 3.0
 - Recombinant Human BACE-2 (rhBACE-2) (Catalog # 4097-ASB)
 - Substrate: MCA-Lys-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH₂ (Catalog # ES010)
 - F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
 - Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute rhBACE-2 to 2 ng/μL in Assay Buffer.
 2. Dilute Substrate to 50 μM in Assay Buffer.
 3. Load 50 μL of 2 ng/μL rhBACE-2 in a plate, and start the reaction by adding 50 μL of 50 μM Substrate. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of 50 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:

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

*Adjusted for Substrate Blank.

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

- Final Assay Conditions**
- Per Well:
- rhBACE-2: 0.1 μg
 - Substrate: 25 μM

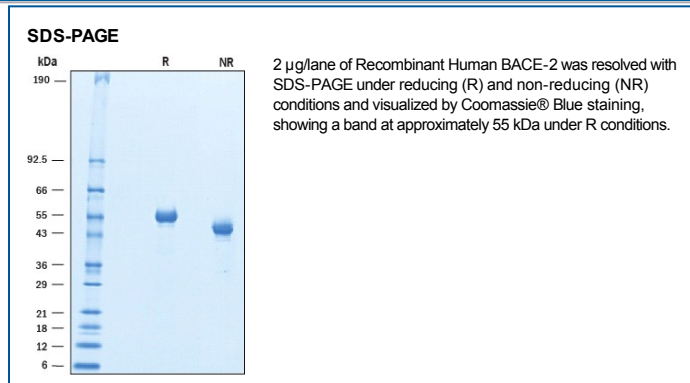
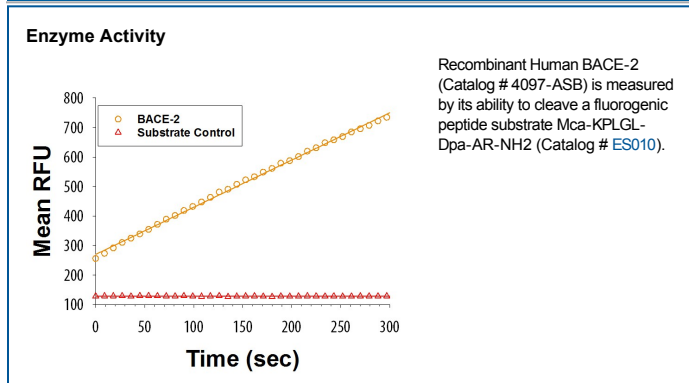
PREPARATION AND STORAGE

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.

DATA



BACKGROUND

BACE-2 (Beta secretase 2) is an aspartic protease that shares 48% sequence identity with BACE-1 in the extracellular catalytic domains. BACE-1 is the putative β secretase for the generation of the A β peptide in neurons (1). BACE-2 differs from BACE-1 in several aspects, including proenzyme activation, substrate preference, transcriptional regulation, and expression pattern (2, 3). Unlike BACE-1, BACE-2 activity does not contribute to Alzheimer's disease pathogenesis (4) but has been shown to play a key role in insulin receptor trafficking in the pancreas where it is expressed in β -cells (5,6). BACE-2 affects glucose tolerance and was suggested as a promising target for improving β -cell function in diabetes (7). Recombinant human BACE-2 was expressed without its C terminal transmembrane and cytosolic domains, resulting in its secretion from NS0 cells.

References:

1. Cai, H. *et al.* (2001) *Nature Neurosci.* **4**:233.
2. Hussain, I. *et al.* (2001) *J. Biol. Chem.* **276**:23322.
3. Ostermann, N. *et al.* (2006) *J. Mol. Biol.* **355**:249.
4. Sun, X. *et al.* (2006) *FASEB J.* **19**:739.
5. Esterhazy, D. *et al.* (2011) *Cell Metab.* **14**:365.
6. Casas, S. *et al.* (2010). *Am. J. Physiol. Endocrinol. Metab.* **299**:E1087.
7. Alcarraz-Vizan, G. *et al.* (2017). *Cell Mol. Life Sci.* **74**:2827.