

Product Specification Sheet

**Beta-Site APP Cleaving Enzyme (BACE/Asp2/Memapsin2) Antibodies**

Cat. # BACE15-R-50 Purified, recombinant human BACE1 protein

**SIZE:** 50 ug

$\beta$ -amyloid (**A $\beta$** ) deposition in the brain is the hallmark of Alzheimer's Disease (**AD**). To initiate A $\beta$  formation,  **$\beta$ -secretase** cleaves APP at the N-terminus of A $\beta$  to release APPs $\beta$  (~100 kDa soluble NT-fragment), and C99, a 12-kDa CT membrane fragment. Alternatively,  **$\alpha$ -secretase** cleaves within the A $\beta$  to prevent the formation of A $\beta$ . Cleavage by  $\alpha$ -secretase produces a soluble N-terminal fragment, APPs $\alpha$ , and a 10-kDa membrane C-terminal fragment, C83. Both C99 and C83 can be further cleaved by  **$\gamma$ -secretase** releasing A $\beta$  and a nonpathogenic p3 peptide, respectively. Recently, **BACE (Beta-site APP Cleaving Enzyme)** has been identified as  **$\beta$ -secretase**. BACE belongs to the family of **Aspartyl proteases (Asp)** also known as **Memapsins**. At least four related Asps, located on chromosome IV and X, have been cloned (**Asp1, Asp2, Asp3, and Asp4**). Human **BACE/Asp2/Memapsin2**, located on chromosome 11, is a transmembrane protein of 501 aa (signal peptide 1-21 aa, a proprotein domain 22-45 aa, 1 TM domain near the CT, and a short cytoplasmic CT-tail of 24 aa; mature protein 46-460 aa). BACE expression was most prominent in most areas of the rat brain and pancreas. It has been localized in the compartments of the secretory pathways.

**Source of Antigen**

The extracellular domain of Human BACE1 (1-460 aa) (1) was expressed as his-tag secreted protein in NSO myeloma cells and purified >95%. The purified BACE-1 protein (cat # BACE15-R-50) exists as two forms, pro and mature, with N-terminal sequences of TQHGI R L P L R and E T D E E P E E P G, respectively. By SDS-PAGE, the apparent mol. Wt. ~ 68 - 70 kDa for the pro form and 65 - 67 kDa for the mature form. Endotoxin content is <1.0 EU/ug.

**Biological Activity:**

Biological activity was measured by the ability of the recombinant protein to cleave a fluorescent peptide substrate Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(Dpn)-Arg-Arg-NH<sub>2</sub> as measured using excitation and emission wavelengths of 320 and 405 nm, respectively. The specific activity, measured with 10  $\mu$ M ES004 and 1  $\mu$ g enzyme in 100  $\mu$ L of 0.1 M sodium acetate, pH 4.0, at room temperature, is > 3.5 pmoles/min/ $\mu$ g. 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.

**Form & Storage**

The protein is supplied at 50 ug/50 ul in liquid buffer (25mM Tris, 0.15 M NaCl, pH 7.5) or powder form. Reconstitute the powder in water or other appropriate buffer in 50 ul (concn 1 mg/ml) or other desired volume but no less than <100 ug/ml. It is advisable to add BSA at 0.1% as a carrier protein to minimize protein losses. The protein is not necessarily sterile but it can be made sterile by filtering through 0.22  $\mu$ m membrane.

Store powder at -20oC for 3-6 months. Reconstituted or liquid protein should be stored in small aliquots at -20oC to -80oC. Avoid repeated freeze and thaw.

**General References:** Vassar R (1999) Science 286, 735-741; Yan R (1999) Nature 402, 533-537; Sinha S (1999) Nature 537-540; Hussain I (1999) Mol. Cell Neurosci. 14, 419-427; Lin X (2000) PNAS 97, 1456-1460

Citations of ADI's antibodies for Beta-site cleaving enzymes (BACE), see updated list at [www.4adi.com/flr/baceflr.html](http://www.4adi.com/flr/baceflr.html)

\*This product is for In vitro research use only.

BACE15-R-50

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