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Product Specification Sheet

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

Cat. # BACE12-P	Mouse BACE/Asp2 Control/blocking Peptide # 2	**SIZE**: 100 ug
Cat. # BACE12-S	Rabbit Anti-Mouse BACE/Asp2 antiserum # 2	**SIZE**: 100 ul
Cat. # BACE12-A	Rabbit Anti-Mouse BACE/Asp2 IgG # 2 (aff pure)	**SIZE**: 100 ug
Cat. # BACE12-C	Purified, recombinant human BACE1 protein control for WB	**SIZE**: 100 ul

β-amyloid **(Aβ)** deposition in the brain is the hallmark of Alzheimer's Disease (**AD**). To initiate Aβ formation, **β-secretase** cleaves APP at the N-terminus of Aβ to release APPsβ (~100 kDa soluble NT-fragment), and C99, a 12-kDa CT membrane fragment. Alternatively, **α-secretase** cleaves within the Aβ to prevent the formation of Aβ. Cleavage by α-secretase produces a soluble N-terminal fragment, APPsα, and a 10-kDa membrane C-terminal fragment, C83. Both C99 and C83 can be further cleaved by **γ-secretase** releasing Aβ and a nonpathogenic p3 peptide, respectively. Recently, **BACE (Beta-site APP Cleaving Enzyme)** has been identified as **β-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 and Antibodies

Antigen	10-aa peptide of mouse BACE/Asp2 Gene Accession # P56818 ; **Designated (BACE12-P or control peptide)** conjugated to KLH
Location	~N-terminal, Extracellular
Ab Host/type	Rabbit, Polyclonal Unpurified antiserum (cat #BACE12-S) Aff pure IgG (**cat #BACE12-A**) purified over antigen-agarose column
2-ab	**Goat Anti-rabbit IgG-HRP** cat # 20320 (AP, biotin, FITC conjugates also available)
-ve control	**# 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as –ve control**

The extracellular domain of Human BACE1 (1-460 aa) was expressed as his-tag protein in E. coli and purified >95%. For **western blot +ve control (Cat # BACE12-C)**, it is supplied in SDS-PAGE sample buffer (reduced). Load ~10 ul/lane to visualize with antibodies (Cat # **BACE12**-S or **BACE12**-A). Store at –20oC to –80oC in suitable aliquots. Do not freeze and thaw. Heat once prior to loading on gels. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **BACE12**-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. This preparation is intended for qualitative purpose and not to serve as standard of known concentration.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 0.05% azide
Reconstitute powder in 100 ul PBS

Affinity pure IgG
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique). An antibody made to BACE12-P epitope has been used to visualize BACE/Asp2 (see Hussain , 1999). Purified BACE1 (Cat #BACE11-C) migrates as 72 and 75 kDa, corresponding to pro and mature BACE1.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry: Not tested**.** We recommend the use of 2:20 ug/ml of affinity pure antibody (1).

Specificity & Cross-reactivity

The mouse BACE12-P sequence is 100% conserved in human and 90% in rat BACE/Asp2. No significant sequence homology exists with BACE2/Aps1, a homolog of BACE, or other Asps. Antibody crossreactivity in various species is not established. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at: www.4adi.com\data\abblock.html).

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)

*This product is for In vitro research use only.

BACE12- S-A-C-P 50422A

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com