ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Neprilysin (NEP) Antibodies

Cat. # NEP11-S	Rabbit Anti-Mouse NEP antiserum #1	**SIZE**: 100 ul
Cat. # NEP11-A	Rabbit Anti-Mouse NEP IgG # 1(aff pure)	**SIZE**: 100 ug
Cat. # NEP11-P	Mouse NEP11 Control/blocking peptide	**SIZE**: 100 ug
Cat. # NEP11-C	Recombinant Mouse NEP protein control for WB	**SIZE**: 100 ug

NEP [variously termed as **neutral endopeptidase-24.11 (NEP), neprilysin, enkephalinase, EC3.4.24.11, common acute lymphoblastic leukemia antigen (CALLA), CD10**] is the key *in vivo* enzyme degrading biopeptides Aβ, substance-P and enkephalin in brain; atrial natriuretic peptide, bradykinin and endothelin-1 in kidney. It is a 97 KDa ectoenzyme (mouse, rat, human 750aa) with a large extracellular domain containing its catalytic site, which can degrade Aβ on cell surface. NEP is ubiquitous but its high expression in brain is restricted to striatum, olfactory tubercle, substantia nigra, choroids plexes, endopeduncular nucleus, pontine nucleus, and cerebellum and in many peripheral tissues, particularly in brush-border membranes. NEP is also expressed in **soluble** form in human plasma and cerebrospinal fluid. NEP can degrade both synthetic and cell secreted $A\beta_{40}$ and $A\beta_{42}$ and may be a good therapeutic target for the treatment of Alzheimer's disease.

Source of Antigen and Antibodies

Antigen	18-aa peptide from Human mouse neprilysin;(gene accession # Q61391) **Designation (NEP11-P, control peptide**) conjugated to KLH; **epitope location ~ N-terminal, Cytoplasmic domain**
Ab Host/type	Rabbit, Polyclonal; Unpurified antiserum (cat # NEP11-S); Aff pure IgG (**cat # NEP11-A**)
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**

Mouse (# NEP11-C) and human (#NEP12-C) NEP proteins (Tyr45-Trp743; total of 699 residues) was expressed in baculovirus cell line Sf21 and purified to >95% purity. The purified protein migrates as ~90 kDa in SDS-PAGE due to glycosylation. **For WB +ve control** (**Cat # NEP11-C**), it is formulated in SDS-PAGE sample buffer (reduced). This preparation is not biologically inactive. It is not suitable for ELISA or other applications where native protein is required. It is supplied in 100 ul/vial. For WB, heat once and load 10 ul/lane and visualize with appropriate antibodies. 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 NEP11-C solution prior to heating and loading on gels. Store frozen in suitable aliquots. Do not freeze, thaw, or hear repeatedly.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied 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 for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:5K-1:50K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence: not tested. We recommend the use of affinity pure antibody at 2-20 ug/ml.

Specificity & Cross-reactivity

The NEP11-P antigenic peptide is conserved in mouse and rat (100%), rabbit (94%), and human (88%) NEP. No significant sequence homology of NEP11-P is seen with NEPLs or other proteins. Antibody reactivity in various species is not known. 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 see detailed protocol at the web site).

General References: Chen CY et al (1997) J. Immunol. 148, 2817; Malfroy B et al (1987) BBRC 144, 59; Bateman RC et al (1989) JBC 264, 6151; Malfroy B et al (1988) J. Exp. Med. 168, 1247; Shipp MA et al (1988) PNAS 85, 4819; Letarte M et al (1988) J. Exp. Med. 168, 1247; Iwata et al. (2001) Science 292, 1550; Ikeda et al. (2000) J. Biol. Chem. 274, 32469

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

NEP11-S-A-P-C 71212A

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