ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Damage-Induced Neuronal Endopeptidase (DINE) Antibodies

Cat. # DINE11-S	Rabbit Anti-Rat DINE antiserum	**SIZE**: 100 ul
Cat. # DINE11-A	Rabbit Anti-Rat DINE IgG (aff pure)	**SIZE**: 100 ug
Cat. # DINE11-P	Rat DINE Control/blocking peptide	**SIZE**: 100 ug

The peripheral nerve regeneration entails sequential changes in the expression of thousands of genes. These changes are necessary to (1) protect the damaged neurons from death, (2) activate the surrounding glial cells, and (3) accelerate the neurite elongation. The nerve regeneration requires a wide range of active proteases including few members of the M-13 metalloprotease family. The M-13 family comprises several zinc-dependent metalloproteases like **(DINE)**, **PHEX**, **KELL**, **ECE**, **XCE**, **neprilysin** (**NEP**) and neprilysin-like proteases (**NEPLs**). The NEPLs (**NEPL-α, NEPL-β, NEPL-γ**) arise from the alternative splicing of a single NEPL gene and are zinc dependent metalloproteases with ~54 % homology to NEP.

DINE (damage-induced neuronal endopeptidase) or Endothelin converting enzyme-like 1 **(ECEL1)** or X-converting enzyme (**Xce**) is a 95 KDa (mouse/rat/human 775 aa, chromosome 2q36-q37), type II integral membrane metalloprotease containing a conserved zinc-binding motif and an ENXADX consensus sequence, consistent with gluzinitin. The aa sequence of DINE is 36 and 32 % identical to ECE-1 and NEP, respectively. But, DINE is devoid of enzyme activity like ECE. Unlike NEP, DINE has no proteolytic activity to Aβ. However, the enzyme can hydrolyze synthetic NEP substrates and thiorphan, EDTA and phosphoramidon inhibit its activity. DINE also inhibits C2-ceramide induced apoptosis in COS-7 cells. Although, the endogenous substrate for DINE is yet to be identified, its proteolytic activity activates, at least in part, free radical scavenging in damaged neurons. The DINE expression is restricted to brain tissue with predominant expression in hypothalamus, large cholinergic cells in striatum and low expression in virtually all regions of brain except in cerebral cortex, hippocampus and cerebellum. DINE, expression is markedly increased in response to optic, spinal sensory, cortical and thalamic nerve injury.

Source of Antigen and Antibodies

Antigen	A 17-aa peptide sequence (**designated DINE11-P or control peptide**), mapping nears the cytoplasmic N-terminus of **rat DINE** (1) was synthesized, coupled to KLH
Ab Host/type	Rabbit, Polyclonal antiserum # **DINE11**-S and IgG, purified over antigen-agarose (Cat # **DINE11**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

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-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:10K-1:100K; 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 DINE11-P control/antigenic peptide is 100% conserved in rat (100%), mouse (94%) and human (88%) DINE. No significant sequence homology of DINE11-P is seen with NEP, NEPLs or ECEs 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 (use 5-10 ug per 1 ul of antiserum or 1 ug of aff pure IgG) to confirm antibody specificity.

General References: (1) Schweizer A et al (1999) JBC 274, 20450-20456; Valdenair O et al (1999) Mol. Brain. Res. 64, 211-221; Valdenaire O et al (2000) Biochem. J. 346, 611-616; Kiryu-Seo S et al (2000) PNAS 97, 4345-4350;

*This product is for In vitro research use only.

Related materials available from ADI

Antibodies: NEP, NEP-alpha, -beta, -gamma, DINE, PHEX.

DINE11-S-A-P 71212A

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