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Antibodies to L-2 Haloacid Dehalogenase (HAD) Family members

Phospho-Specific Cytosolic IMP/GMP specific 5'Nucleotidase II (Cat # PCNII-140AP)

Various cytotoxic nucleoside analogs (NA) have been employed in the treatment of hematologic malignancies. These NA include pyrimidine analog cytosine arabinoside (ara-c) and purine analogs, cladribine and fludarabine. These NA become therapeutically effective only after phosphorylation to the triphosphate level. The 5'-nucleotidases (5'-NTs) dephosphorylate the monophosphate form of NA and, therefore, may affect the pharmacological activity of these antimetabolites. Several 5'-NTs attached to membranes or present in the cytosol or in mitochondria. There are two cytosolic cyclic 5' nucleotidases are cN-II and dNT-1 and one mitochondrial (dNT-2) nucleotidase known to be present in the cells. cN-I plays a significant role in AMP breakdown to adenosine whereas cN-II breaks down IMP to inosine and GMP to guanosine. The primary sequence of cN-I is unrelated to cN-II or ecto-5' nucleotidase (e-N). The tissue distribution of cN-I cN-II is different, cN-I is found only in vertebrate heart where as cN-II is expressed in heart, brain and muscle. Sequence alignments of cytosolic 5'-nucleotidase, along with other nucleotidases, suggest that cytosolic 5' nucleotidase belong to a large superfamily of hydrolases with different substrate specificities and functional roles. Overproduction of cN-II could lead to resistance against anti-cancer drugs based on purine analogs. cN-II is Mg²⁺-dependent, regulated and stabilized by several factors such as allosteric effectors ATP and 2,3-DPG, although these are not directly involved in the reaction stoichiometry (2).

IMP and GMP and its derivatives specific cytosolic 5'-nucleotidase (cNII) acts through the formation of a phosphoenzyme intermediate. Phosphate either released leading to 5'-mononucleotide hydrolysis or transferred to an appropriate nucleoside acceptor, giving rise to a mononucleotide interconversion. Chemical reagents specifically modifying aspartate and glutamate residues inhibit the enzyme, and this inhibition is partially prevented by cN-II substrates and physiological inhibitors (3). Site-directed mutagenesis experiments confirmed the essential role of Asp-52 in the catalytic machinery of the enzyme and suggested also that Asp-54 assists in the formation of the acyl phosphate species. There exist a putative phosphorylation site on the cNII near C-terminal end of the protein. FabGennix Int. Inc., has also produced a phospho-specific antibody to phospho-serine residue at this site (phosphorylation site: ILFRSG(Sp)RQTLf). The cNII exist as monomer (40 kDa) and multimeric forms in various tissues.

Anti-Phospho-cNII-selective antibodies were generated using kinase site (SLFRSG SR(p)QTLF, the peptide was post-synthetically modified to achieve the desired antigenicity. The affinity purified version of this antibody (PcNII-140AP) was isolated from immobilized antigen based affinity chromatography and adsorbed on non-phospho are represented as pure IgG fractions stabilized in antibody stabilization buffer. The mono-epitope specific polyclonal antibody (cNII-101AP) strongly label a 40-42 kDa cNII and its multimeric forms in PC-cNII samples. A phospho-specific antibody to cNII is also available from FabGennix Int. Inc., (cat. # cNII-140AP). The cNII antibodies can be conjugated as HRP or alkaline conjugates for IHC, Confocal, WB analyses at a nominal fee. FabGennix Inc. will also conjugate antibodies with fluorescent probes upon request at extra charge. FabGennix Inc. also provides antibodies against other diagnostic/neomarkers, the list of these antibodies can be obtained at www.FabGennix.com under Antibodies to diagnostic markers. Limited quantities of antigens are also available. Please enquire for their availability before ordering.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	Price
PCNII-140AP	Rabbit	Affinity purified Phospho-cNII antibodies	R, M, H	100 µg	285
P-PcNII	n/a	Antigenic blocking peptide for cNII-101AP	n/a	250 ug	145
P-cNII	n/a	Antigenic blocking peptide for PcNII-140AP	n/a	250 ug	115
PC-cNII	n/a	Western blot positive control for cNII	n/a	5 appl	195

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; * not all variants are labeled equally

Immunogen: Synthetic peptides corresponding to cNII sequence. For phospho-cNII antibodies the peptide was (slf rsg s(p)rgt lf. Serine 7 was phosphorylated.

Concentration: PCNII-140AP = 0.75-0.85 mg/ml of antibody stabilization buffer

Applications: Antibody PCNII-140AP is ideal for WB; IMM. IHC and confocal applications are not yet determined. The cross species reactivity and its applications in other protocols is not determined. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay in his/her laboratory. Suggested Dilutions: WB: > 1:500; IMM & i.p pull-down assays: n.d; IHC for PCNII-140AP n.d

Protocols: Standard protocol for various applications (WB; IMM and IHC) of this antibody will be provided upon request, +however, FabGennix Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

References:

- Galmarini CM, Jordheim L, Dumontet C Leuk Lymphoma. 2003 Jul;44(7):1105-11.
- Brettonnet AS, Jordheim LP, Dumontet C, Lancelin JM. FEBS Lett. 2005 Jun 20;579(16):3363-8.
- Allegrini S, Scaloni A, Ferrara L, et. Al., J Biol Chem. 2001 Sep 7;276(36):33526-32. Epub 2001 Jun 29.

* For users who may require large amounts of PCNII-140AP, please enquire about bulk material discounts.

This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

043106-0020SF1001Z-rev10.00

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