

APB250Hu01 100µg

Active 5'-Nucleotidase, Ecto (NT5E)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr., 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Leu29~Thr500 Tags: N-terminal His-tag

Purity: >92%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl

and 5% trehalose.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.4

Predicted Molecular Mass: 53.6kDa

Accurate Molecular Mass: 53kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

CVNASRCMGG VARLFTKVQQ IRRAEPNVLL LDAGDQYQGT IWFTVYKGAE VAHFMNALRY DAMALGNHEF DNGVEGLIEP LLKEAKFPIL SANIKAKGPL ASQISGLYLP YKVLPVGDEV VGIVGYTSKE TPFLSNPGTN LVFEDEITAL QPEVDKLKTL NVNKIIALGH SGFEMDKLIA QKVRGVDVVV GGHSNTFLYT GNPPSKEVPA GKYPFIVTSD DGRKVPVVQA YAFGKYLGYL KIEFDERGNV ISSHGNPILL NSSIPEDPSI KADINKWRIK LDNYSTQELG KTIVYLDGSS QSCRFRECNM GNLICDAMIN NNLRHTDEMF WNHVSMCILN GGGIRSPIDE RNNGTITWEN LAAVLPFGGT FDLVQLKGST LKKAFEHSVH RYGQSTGEFL QVGGIHVVYD LSRKPGDRVV KLDVLCTKCR VPSYDPLKMD EVYKVILPNF LANGGDGFQM IKDELLRHDS GDQDINVVST YISKMKVIYP AVEGRIKFST

[ACTIVITY]

5'-Nucleotidase, Ecto (NT5E), also known as ecto-5'-nucleotidase or CD73, is an enzyme catalyzing thehydrolysis of nucleoside-5'-monophosphates to nucleosides and inorganic phosphate. The enzyme is a dimer composed of 2 identical 70kD subunits bound by a glycosyl phosphatidyl inositol linkage to the external face of the plasma membrane. NT5E is a marker of lymphocyte differentiation that has functions independent of its catalytic activity, such as T-cell activation and cell-cell adhesion. Other forms of 5-prime nucleotidase exist in the cytoplasm and lysosomes and can be distinguished from NT5E by their substrate affinities, requirement for divalent magnesium ion, activation by ATP, and inhibition by inorganic phosphate. The enzyme is widely distributed in human and animal tissues. Besides, AF4/FMR2 Family, Member 1 (AFF1) has been identified as an

interactor of NT5E thus a binding ELISA assay was conducted to detect the interaction of recombinant human NT5E and recombinant human AFF1. Briefly, NT5E were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to AFF1-coated microtiter wells and incubated for 2h at 37° C. Wells were washed with PBST and incubated for 1h with anti-NT5E pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37° C. Finally, add 50μ L stop solution to the wells and read at 450nm immediately. The binding activity of of NT5E and AFF1 was shown in Figure 1, and this effect was in a dose dependent manner.

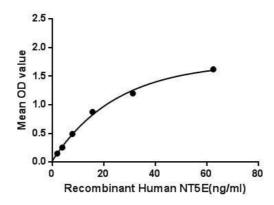


Figure 1. The binding activity of NT5E with AFF1

[IDENTIFICATION]

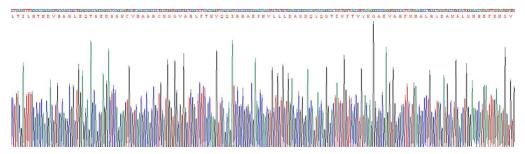


Figure 2. Gene Sequencing (extract)

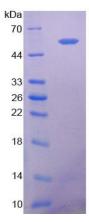


Figure 3. SDS-PAGE

Sample: Active recombinant NT5E, Human

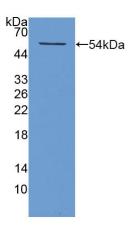


Figure 4. Western Blot

Sample: Recombinant NT5E, Human;

Antibody: Rabbit Anti-Human NT5E Ab (PAB250Hu01)