APB250Hu01 10μg
Active 5'-Nucleotidase, Ecto (NT5E)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st 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^{o}C for one month.
Aliquot and store at -80^{o}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 37ºC 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]

LT ILHTNDVHSR LEQTSEDSSK
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℃. 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℃. Finally, add 50μL 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.

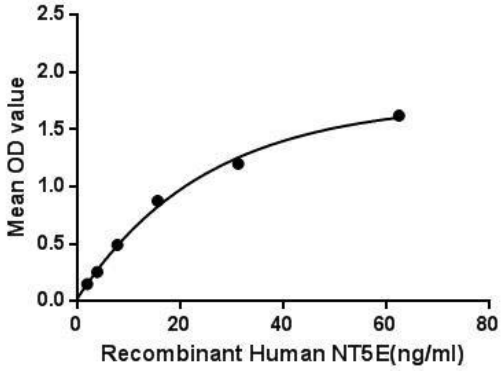

Figure 1. The binding activity of NT5E with AFF1

[IDENTIFICATION]

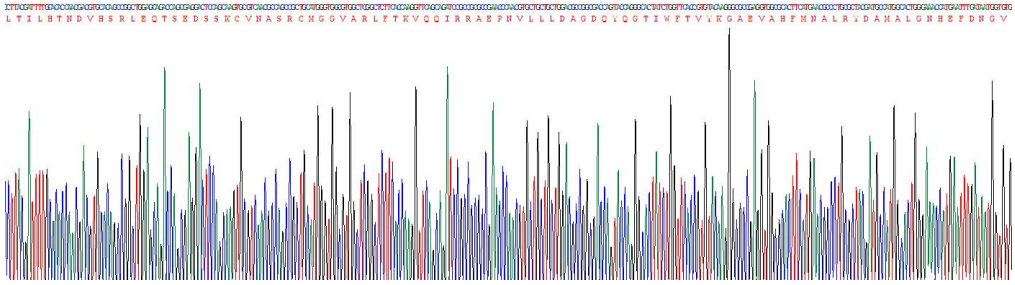

Figure 2. Gene Sequencing (extract)

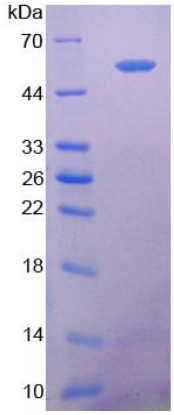

Figure 3. SDS-PAGE

Sample: Active recombinant NT5E, Human

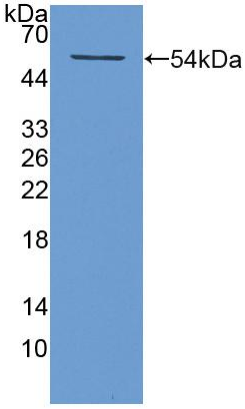

Figure 4. Western Blot

Sample: Recombinant NT5E, Human;

Antibody: Rabbit Anti-Human NT5E Ab (PAB250Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.