



APB127Hu01 100µg

Active Deoxyribonuclease I (DNASE1)

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: Gly19~Ala259

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: 5.0

Predicted Molecular Mass: 28.3kDa

Accurate Molecular Mass: 28kDa 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]

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GA VSLKIAAFNI QTFGETKMSN ATLVSYIVQI
LSRYDIALVQ EVRDSHLTAV GKLLDNLNQD APDTYHYVVS EPLGRNSYKE
RYLFVYRPDQ VSAVDSYYYD DGCEPCGNDT FNREPAIVRF FSRFTEVREF
AIVPLHAAPG DAVAEIDALY DVYLDVQEKW GLEDVMLMGD FNAGCSYVRP
SQWSSIRLWT SPTFQWLIPD SADTTATPTH CAYDRIVVAG MLLRGAVVPD
SALPFNFQA
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[ACTIVITY]

Deoxyribonuclease I (usually called DNase I) is a nonspecific endonuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, yielding 5'-phosphate-terminated polynucleotides with a free hydroxyl group on position 3', on average producing tetranucleotides. It acts on single-stranded DNA, double-stranded DNA, and chromatin. DNase I can be activated by bivalent metals such as Mg²⁺ and Ca²⁺. This endonuclease enzyme is common reagents used in biochemical methods requiring digestion of DNA and recovery of RNA, or where DNA is to be removed without affecting structural proteins or enzymes. For example, DNase I is frequently used to remove template DNA following in vitro transcription, and to remove contaminating DNA in total RNA preparations (especially those from transfected cells that may contain plasmid DNA), used for ribonuclease protection assays, cDNA library contraction, and RT-PCR. Besides, Actin Beta (ACTb) has been identified as an interactor of DNase I, thus a binding ELISA assay was conducted to detect the interaction of recombinant human DNase I and recombinant human ACTb. Briefly, DNase I were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were

then transferred to ACTb-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-DNase I 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 50µL stop solution to the wells and read at 450nm immediately. The binding activity of DNase I and ACTb was shown in Figure 1, and this effect was in a dose dependent manner.

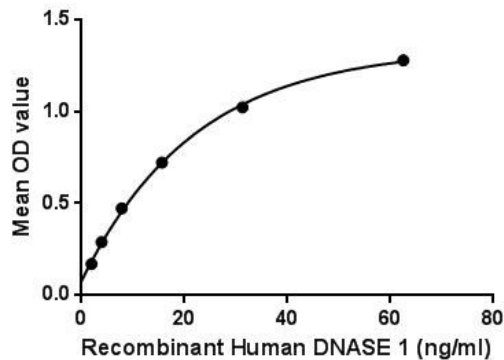


Figure 1. The binding activity of DNASE I with ACTb.

[IDENTIFICATION]

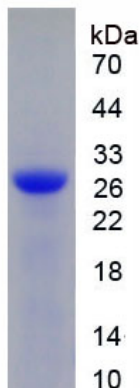


Figure 2. SDS-PAGE

Sample: Active recombinant DNASE1, Human

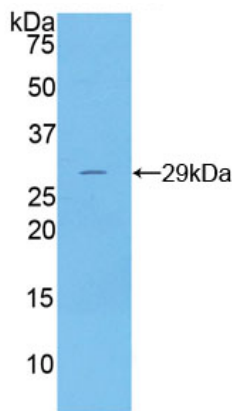


Figure 3. Western Blot

Sample: Recombinant DNASE1, Human;

Antibody: Rabbit Anti-Human DNASE1 Ab (PAB127Hu01)