

APA711Hu01 100μg

Active Heparanase (HPA)

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: Pro400~lle543
Tags: N-terminal His-tag

Purity: >97%

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

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: 9.9

Predicted Molecular Mass: 17.6kDa

Accurate Molecular Mass: 20kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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]

P

LPDYWLSLLF KKLVGTKVLM ASVQGSKRRK LRVYLHCTNT DNPRYKEGDL TLYAINLHNV TKYLRLPYPF SNKQVDKYLL RPLGPHGLLS KSVQLNGLTL KMVDDOTLPP LMEKPLRPGS SLGLPAFSYS FFVIRNAKVA ACI

[ACTIVITY]

Heparanase(HPA), also known as HPSE, is an enzyme that acts both at the cell-surface and within the extracellular matrix to degrade polymeric heparan sulfate molecules into shorter chain length oligosaccharides. Besides, Epithelial Cell Transforming Sequence 2 (ECT2) has been identified as an interactor of HPA, thus a binding ELISA assay was conducted to detect the interaction of recombinant human HPA and recombinant human ECT2. Briefly, HPA were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µL were then transferred to ECT2-coated microtiter wells and incubated for 2h at 37 $^{\circ}\!\!\!\!^{\circ}$. Wells were washed with PBST and incubated for 1h with anti-HPA 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 $^{\circ}\!\!\!\!^{\circ}\!\!\!\!^{\circ}$. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of HPA and ECT2 was shown in Figure 1, and this effect was in a dose dependent manner.

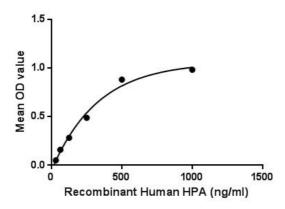


Figure 1. The binding activity of HPA with ECT2

[IDENTIFICATION]

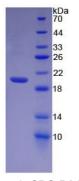


Figure 2. SDS-PAGE

Sample: Active recombinant HPA, Human

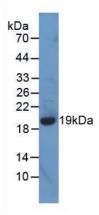




Figure 3. Western Blot

Sample: Recombinant HPA, Human;

Antibody: Rabbit Anti-Human HPA Ab (PAA711Hu01)

[IMPORTANT NOTE]

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