APA711Hu61 10µg Active Heparanase (HPSE) Organism Species: *Homo sapiens* (Human) *Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression. Host: 293F cell Residues: Gln36~lle543 Tags: N-terminal His-tag **Purity:** >95% **Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 5%Trehalose. Original Concentration: 50µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.5 Predicted Molecular Mass: 59.2kDa Accurate Molecular Mass: 75kDa as determined by SDS-PAGE reducing conditions. Phenomenon explanation: The possible reasons that the actual band size differs from the predicted are as follows: 1. Splice variants: Alternative splicing may create different sized proteins from the same gene. 2. Relative charge: The composition of amino acids may affects the charge of the protein. 3. Post-translational modification: Phosphorylation, glycosylation, methylation etc. 4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form. 5. Polymerization of the target protein: Dimerization, multimerization etc.

[<u>USAGE</u>]

Reconstitute in ddH₂O to a concentration \leq 0.1mg/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.

[<u>SEQUENCE</u>]

QDVVDLDFFTQEPLHLVSPSFLSVTIDANLATDPRFLILLGSPKLRTLARGLSPAYLRFGGTKTDFLIFDPKKESTFEERSYWQSQVNQ DICKYGSIPPDVEEKLRLEWPYQEQLLLREHYQKKFKNSTYSRSSVDVLYTFANCSGLDLIFGLNALLRTADLQWNSSNAQLLLDYCSS KGYNISWELGNEPNSFLKKADIFINGSQLGEDFIQLHKLLRKSTFKNAKLYGPDVGQPRRKTAKMLKSFLKAGGEVIDSVTWHHYYLNG RTATKEDFLNPDVLDIFISSVQKVFQVVESTRPGKKVWLGETSSAYGGGAPLLSDTFAAGFMWLDKLGLSARMGIEVVMRQVFFGAGNY HLVDENFDPLPDYWLSLLFKKLVGTKVLMASVQGSKRRKLRVYLHCTNTDNPRYKEGDLTLYAINLHNVTKYLRLPYPFSNKQVDKYLL RPLGPHGLLSKSVQLNGLTLKMVDDQTLPPLMEKPLRPGSSLGLPAFSYSFFVIRNAKVAACI

[<u>ACTIVITY</u>]

Heparanase (HPSE) selectively cleaves heparan sulfate at specific sites on heparan sulfate proteoglycans (HSPGs). HPSE facilitates cell migration associated with metastasis, wound healing and inflammation. An increase in its activity is associated with an increase in VEGF activity, which further enhances angiogenesis. HPSE also enhances shedding of syndecans and increases endothelial invasion and angiogenesis in myelomas. It acts as a procoagulant by increasing the generation of activation factor X in the presence of tissue factor and activation factor VII. In addition, it increases cell adhesion to the extracellular matrix (ECM), independent of its enzymatic activity.

HPSE is highly expressed in placenta and spleen and weakly expressed in lymph node, thymus, peripheral blood leukocytes, bone marrow, endothelial cells, fetal liver and tumor tissues. The enzyme activity of recombinant human HPSE was assayed in an ELISA format using non-reducing end biotinylated heparan sulfate on recombinant human syndecan 4 as a substrate. Briefly, combining 10 µL of different concentrations of rhHPSE with 10 μ L of the biotinylated-syndecan 4 in a vial (10 μ L of assay buffer and 10 μ L of biotinylated-syndecan 4 as a negative control) and incubated for 2h at 37 $^\circ$ C. After incubation, 220 µL of PBS, with 1% BSA (pH7.4) was added to each reaction, then loading 100 ul of each sample onto the anti-syndecan 4 pAb-coated microtiter wells and incubated for 1h at 37 °C. Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 °C. Finally, add 50 µl stop solution to the wells and read at 450nm immediately. As a result, 62.5 ng of recombinant human HPSE digestion will result in >50% of OD reduction compared with the negative control.

[IDENTIFICATION]

Figure 1. SDS-PAGE

Sample: Active recombinant HPSE, Human

[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.