

**DESCRIPTION**

**Source** Chinese Hamster Ovary cell line, CHO-derived human Heparanase/HPSE protein  
Gln36-Ile543 with N-terminal 6-His tag  
Accession # AF144325  
The linker peptide from Ser110-Gln157 is removed by protease treatment

**N-terminal Sequence Analysis** His & K158

**Predicted Molecular Mass** 43 kDa & 9 kDa

**SPECIFICATIONS**

**SDS-PAGE** 50-60 kDa & 7-9 kDa, reducing conditions

**Activity** Measured by its ability to release biotinylated heparan sulfate from Recombinant Human Syndecan-4 (Catalog # 2918-SD).  
20 ng of Recombinant Human Active Heparanase/HPSE digestion will result in >50% of OD reduction compared with the Negative Control, as measured under the described conditions.

**Endotoxin Level** <1.0 EU per 1 µg of the protein by the LAL method.

**Purity** >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation** Supplied as a 0.2 µm filtered solution in Tris, NaCl and E64. See Certificate of Analysis for details.

**Activity Assay Protocol**

- Materials**
- Assay Buffer: 50 mM Sodium Acetate, pH 5.0
  - Recombinant Human HPSE (rhHPSE) (7570-GH)
  - HPSE Substrate (Catalog # ES020)
  - Human Syndecan-4 DuoSet Kit (Catalog # DY2918)
  - 96-well Clear Plate (Catalog # DY990)
  - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

And the following materials that are routinely used in ELISA:

- Coating Buffer (Catalog # DY006)
- Wash Buffer (25X) (Catalog # WA126)
- Reagent Diluent (10X) (Catalog # DY995)
- Substrate Reagent Pack (Catalog # DY999)
- Stop Solution (Catalog # DY994)

- Assay**
1. Prepare an ELISA plate by following the DuoSet kit protocol.
  2. Dilution factor determination:
    - a. Dilute the HPSE Substrate stock 100-fold in Reagent Diluent (this will be the first dilution point).
    - b. Further prepare a 2-fold serial dilution series of the above diluted HPSE Substrate with Reagent Diluent for 6 points.
    - c. Load 100 µL of each point onto the prepared ELISA plate in duplicate. Load 100 µL of Reagent Diluent to 2 separate wells for blank control.
    - d. Cover the plate and incubate at room temperature for 2 hours.
    - e. Follow the DuoSet Assay Procedure from step 4 to step 9 to complete the assay.
    - f. Determine the dilution factor (n) that achieves an OD between 1.8-3.0.
  3. rhHPSE Activity Detection:
    - a. Dilute the HPSE Substrate stock by n/10-fold in Assay Buffer, dilute rhHPSE to 2 µg/mL in assay buffer.
    - b. Combine 10 µL of rhHPSE with 10 µL of the diluted HPSE Substrate in a vial. For negative control, combine 10 µL of Assay Buffer and 10 µL of the diluted HPSE Substrate in a vial.
    - c. Incubate reactions and negative control at 37 °C for 2 hours.
    - d. After incubation, heat all reactions and negative control at 95 °C for 2 minutes to inactivate rhHPSE.
    - e. Add 220 µL of Reagent Diluent to each reaction and negative control. Mix well.
    - f. Load 100 µL of each sample onto the prepared ELISA plate in duplicate.
    - g. Cover the plate and incubate for 2 hours at room temperature.
    - h. Follow the DuoSet Assay Procedure from step 4 to step 9 to complete the assay.
  4. Calculate % OD reduction compared with the negative control:  

$$[1 - (\text{OD of rhHPSE sample} / \text{OD of negative control})] \times 100 = \% \text{ OD reduction}$$

**Final Assay Conditions** Per Reaction:  
rhHPSE: 20 ng

**PREPARATION AND STORAGE**

**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

**BACKGROUND**

Heparanase (HPSE) selectively cleaves heparan sulfate at specific sites on heparan sulfate proteoglycans (HSPGs) (1, 2, 3, 4). The enzyme is synthesized as an inactive 65 kDa proenzyme that is secreted via the Golgi apparatus and associates with the cell membrane through interaction with HSPGs (5). It is then endocytosed and transferred to lysosomes (6) where cathepsin L activates it by removing an internal inhibitory peptide, forming a heterodimer composed of an 8 kDa and a 50 kDa subunit (7, 8). Under certain stimuli, the active enzyme is transferred back to the cell surface, where it participates in extracellular matrix degradation and remodeling (9). HPSE facilitates cell migration associated with metastasis, wound healing and inflammation (10). An increase in its activity is associated with an increase in VEGF activity, which further enhances angiogenesis (11). HPSE also enhances shedding of syndecans and increases endothelial invasion and angiogenesis in myelomas (12). It acts as a procoagulant by increasing the generation of activation factor X in the presence of tissue factor and activation factor VII (13). In addition, it increases cell adhesion to the extracellular matrix (ECM), independent of its enzymatic activity (14). 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 (15). The enzyme activity of recombinant human HPSE was assayed in an ELISA format using non-reducing end biotinylated heparan sulfate on recombinant syndecan 4 as a substrate (16).

**References:**

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