

#### DESCRIPTION

**Source** *E. coli*-derived *p. heparinus* Heparinase II protein  
Ala26-Arg772, with an N-terminal Met and 6-His tag  
Accession # YP\_003092674

**N-terminal Sequence Analysis** Met

**Predicted Molecular Mass** 86 kDa

#### SPECIFICATIONS

**SDS-PAGE** 66-75 kDa, reducing conditions

**Activity** Measured by its ability to liberate oligosaccharides from heparin.  
The specific activity is >750 pmol/min/μg, 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 and NaCl. See Certificate of Analysis for details.

#### Activity Assay Protocol

- Materials**
- Assay Buffer: 100 mM Tris, pH 7.5
  - Recombinant *P. heparinus* Heparinase II (rPhHeparinase II) (Catalog # 6336-GH)
  - Substrate: Heparin (Tocris, Catalog # 2812), 20 mg/mL stock in deionized water
  - 96 well clear UV-transparent microplate (Corning, Catalog # 3635)
  - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute rPhHeparinase II to 20 ng/μL in Assay Buffer.
  2. Dilute Substrate to 3.0 mg/mL in Assay Buffer.
  3. Load into a plate 50 μL of the diluted rPhHeparinase II, and start the reaction by adding 50 μL of 3.0 mg/mL Substrate. Include a Substrate Blank containing 50 μL of Assay Buffer and 50 μL of 3.0 mg/mL Substrate.
  4. Read in kinetic mode for 5 minutes at an absorbance of 232 nm.
  5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/μg)} = \frac{\text{Adjusted } V_{\max}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme (μg)}}$$

\*Adjusted for Substrate Blank

\*\*Using the extinction coefficient 3800 M<sup>-1</sup>cm<sup>-1</sup>

\*\*\*Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

- Final Assay Conditions**
- Per Well:
- rPhHeparinase II: 1.0 μg
  - Substrate: 1.5 mg/mL

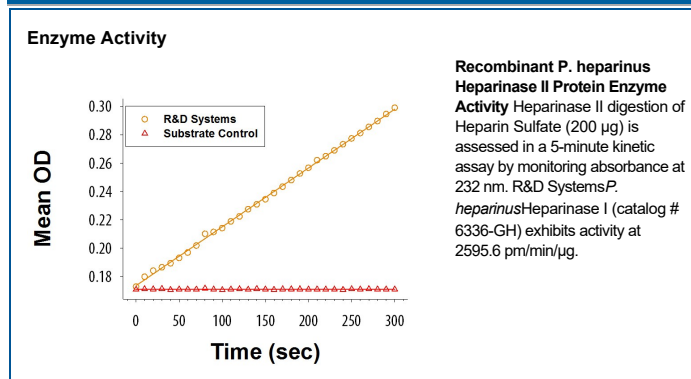
#### PREPARATION AND STORAGE

**Shipping** The product is shipped with dry ice or equivalent. 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

#### DATA



#### BACKGROUND

Heparan sulfate is a sulfated glycosaminoglycan with the repeating disaccharide units of -4HexA1,4GlcNAcβ1-. It is usually attached to the protein cores of proteoglycans found on cell membrane and extracellular matrix, where it binds to a variety of protein ligands and regulates a wide range of biological activities, including developmental processes, angiogenesis, blood coagulation and tumor metastasis (1, 2). Heparan sulfate has a domain structure containing sulfated regions interspaced with less or non-sulfated regions (3, 4). Heparin shares the backbone structure with heparan sulfate but contains no non-sulfated regions. Heparinases are a family of lyases that release unsaturated oligosaccharides from heparin and heparan sulfate upon digestion (5). Heparinase I recognizes highly sulfated regions and is more specific for heparin. Heparinase II digests both heparin and heparan sulfate. Heparinase III prefers less-sulfated regions and is active only on heparan sulfate (6, 7).

#### References:

1. MacArthur, J. M. *et al.* (2007) *J. Clin. Invest.* **117**:153.
2. Esko, J. D. and Selleck, S. B. (2002) *Annu. Rev. Biochem.* **71**:435.
3. Maccarana, M. *et al.* (1996) *J. Biol. Chem.* **271**:17804.
4. Linker, A. and Hovingh, P. (1975) *Biochim. Biophys. Acta.* **385**:324.
5. Linker, A. and Hovingh, P. (1965) *J. Biol. Chem.* **240**:3724.
6. Su, H. *et al.* (1996) *Appl. Environ. Microbiol.* **62**:2723.
7. Hovingh, P. and Linker, A. (1970) *J. Biol. Chem.* **245**:6170.