Recombinant *P. heparinus* Heparinase II

Catalog Number: 6336-GH

DESCRIPTION

Source	*E. coli*-derived 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 under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 100 mM Tris, pH 7.5
- Recombinant *P. heparinus* Heparinase II (r*Ph*Heparinase 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 r*Ph*Heparinase 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 r*Ph*Heparinase 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:

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

*Adjusted for Substrate Blank
**Using the extinction coefficient 3800 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

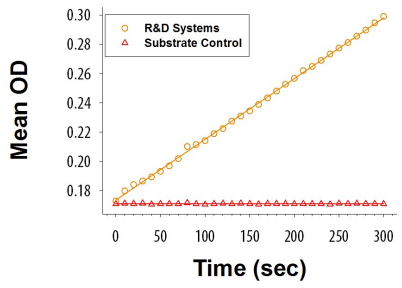
- r*Ph*Heparinase II: 1.0 µg
- Substrate: 1.5 mg/mL

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

Enzyme Activity



Heparinase II digestion of Heparin Sulfate (200 µg) is assessed in a 5-minute kinetic assay by monitoring absorbance at 232 nm. R&D Systems *P. heparinus* Heparinase I (catalog # 6336-GH) exhibits activity at 2595.6 pm/min/µg.

Recombinant *P. heparinus* Heparinase II

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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:

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