Recombinant Human Exostosin 1/2 Heterodimer

Catalog Number: 8567-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Exostosin 1/2 Heterodimer protein Arg29-Leu746 with a C-terminal 2-His and HA tag (EXT1) & Ser53-Leu718 with a C-terminal 6-His tag (EXT2) Accession # Q16394 (EXT1) & Q93063 (EXT2)
N-terminal Sequence Analysis	Arg29 (EXT1) & Ser53 (EXT2)
Predicted Molecular Mass	84 kDa (EXT1) & 77 kDa (EXT2)

SPECIFICATIONS

SDS-PAGE	69-85 kDa, reducing conditions
Activity	Measured by its ability to transfer GlcNAc and GlcA from donor substrates to heparan sulfate. The specific activity is >225 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Glycosyltransferase Activity Kit (Catalog # EA001)
- Assay Buffer: 25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, 5 mM $MnCl_2$, pH 7.0
- Recombinant Human Exostosin 1/2 Heterodimer (rhEXT1/2) (Catalog # 8567-GT)
- UDP-GlcA (Sigma, Catalog # U5625), 10 mM stock in deionized water
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol/50% deionized water
- Heparan Sulfate (Celsus Labs, Catalog # HO-03101 or HO-3105), 50 mg/mL stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
2. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare a reaction mixture containing 1 mM UDP-GlcA, 1 mM UDP-GlcNAc, 8 mg/mL Heparan Sulfate, and 4 µg/mL Coupling Phosphatase 1 in Assay Buffer.
4. Dilute rhEXT1/2 to 4 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of 4 µg/mL rhEXT1/2 into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
8. Seal plate and incubate at 37 °C for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhEXT2+rhEXT1: 0.1 µg
- UDP-GlcA: 0.5 mM
- UDP-GlcNAc: 0.5 mM
- Heparan Sulfate: 200 µg
- Coupling Phosphatase 1: 0.1 µg

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.

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BACKGROUND

Exostosin (EXT) family of glycosyltransferases are involved in heparan sulfate (HS) and heparin biosynthesis (1). Mutations of these enzymes are the causes of hereditary multiple exostoses, which are characterized by formation of numerous cartilage-capped, benign bone tumors (osteocartilaginous exostoses or osteochondromas) that are often accompanied by skeletal deformities and short stature (2). In a small percentage of cases exostoses have exhibited malignant transformation resulting in an osteosarcoma or chondrosarcoma (3). Five members of this family have been cloned to date: EXT1, EXT2, EXTL1, EXTL2, and EXTL3. EXT1 and EXT2 are bifunctional enzymes with both β-1,4-glucuronyltransferase and α-1,4-N-acetylglucosaminyltransferase activities that elongate HS chains with alternating β-1,4-GlcA and α-1,4-GlcNAc residues on the non-reducing end (4). EXT1 is an active enzyme, but the hetero-dimer of EXT1 and EXT2 has increased stability and activity (1). The enzyme activity is measured using a phosphatase-coupled assay (5).

References:

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5. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

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