Recombinant *P. multocida* Hyaluronan Synthase

Catalog Number: 9585-GT

DESCRIPTION

Source	*E. coli*-derived Met1-Ile703, with C-terminal 6-His tag Accession # Q7BLV3
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	81 kDa

SPECIFICATIONS

SDS-PAGE	74 kDa, reducing conditions
Activity	Measured by its ability to transfer GlcNAc and GlcA from donor substrates to hyaluronan. The specific activity is >80 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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

- Glycosyltransferase Activity Kit (Catalog # EA001)
- 10X Assay Buffer (supplied in kit): 250 mM Tris, 100 mM $CaCl_2$, pH 7.5
- $MnCl_2$ (supplied in kit): 100 mM
- Recombinant *P. multocida* Hyaluronan Synthase (r*P.multocida* HAS) (Catalog # 9585-GT)
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol
- UDP-GlcA (Sigma, Catalog # U5625), 10 mM stock in deionized water
- Hyaluronan (Ultra-Low MW) (Catalog # GLR003), 100 mg/mL in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare 1X Assay Buffer containing 10 mM $MnCl_2$ by combining 10X stocks and diluting 10 fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Prepare reaction mixture containing 0.4 mM UDP-GlcNAc, 0.4 mM UDP-GlcA, 8 mg/mL Hyaluronan, and 4 µg/mL Coupling Phosphatase 1 in 1X Assay Buffer.
5. Dilute r*P.multocida* HAS to 16 ng/µL in 1X Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
7. Load 25 µL of 16 ng/µL r*P.multocida* HAS into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
8. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
9. Seal plate and incubate at 37 °C for 20 minutes.
10. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
11. Add 100 µL of deionized water to all wells. Mix briefly.
12. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate for 20 minutes at room temperature.
13. Read plate at 620 nm (absorbance) in endpoint mode.
14. 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:

- r*P.multocida* HAS: 0.4 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-GlcNAc: 0.2 mM
- UDP-GlcA: 0.2 mM
- Hyaluronan: 200 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

Hyaluronan (HA) is a polysaccharide chain composed of repeating β4GlcUA-β3GlcNAc disaccharide units with molecular weights generally ranging from ~10^4 to 10^7 Da in vertebrates and bacteria (1, 2). In animals, HA plays structural, recognition and signaling roles. Certain pathogenic bacteria, namely *Streptococcus* Group A and C and *Pasteurella multocida* Type A, utilize extracellular HA polysaccharide capsules to avoid host defenses and to increase virulence. It is now recognized that HA of different sizes can have dramatically different effects on cellular behavior and growth (3, 4), and vertebrates may be able to control HA size *in vivo* by differential expression of biosynthetic enzymes (5). The *Pasteurella multocida* HA synthase enzyme, pmHAS, catalyzes the synthesis of HA polymer by alternative addition of GlcNAc and GlcA residues to the nonreducing terminus of HA using the donor substrates UDP-GlcNAc and UDP-GlcA (6). The enzyme can also elongate exogenous HA oligosaccharide acceptors *in vitro* (7, 8), therefore can be used for non-reducing end labeling of HA. The enzymatic activity of pmHAS was determined using a phosphatase-coupled assay (9).

References:

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