Goat Keratan Sulfate Proteoglycans

Catalog Number: 8618-KS

DESCRIPTION

Source	Goat cornea This product presumably contains lumican, keratocan and mimecan.

SPECIFICATIONS

SDS-PAGE	30-300 kDa, reducing conditions
Activity	Measured by its ability to act as a substrate for Recombinant *F. keratolyticus* Endo-beta-galactosidase. >90% of Keratan Sulfate Proteoglycans can be cleaved under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE with silver staining.
Formulation	Supplied as a 0.2 µm filtered solution in deionized water. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Labeling Buffer: 25 mM MES, 0.5% (v/v) Triton® X-100, 2.5 mM $MgCl_2$, 2.5 mM $MnCl_2$, 1.25 mM $CaCl_2$, 0.75 mg/mL BSA, pH 7.0
- Assay Buffer: 0.1 M MES, pH 6.0
- Gel Running Buffer: 40 mM Tris, 1 mM EDTA, adjust to pH 8.0 with acetic acid
- Recombinant *F. keratoyticus* Endo-β-Galactosidase (rF.k. Endo-β-galactosidase) (Catalog # 8620-GH)
- Goat Keratan Sulfate Proteoglycans (Catalog # 8618-KS)
- Recombinant Human Carbohydrate Sulfotransferase 1/CHST1 (rhCHST-1) (Catalog # 5316-ST)
- 8% SDS-PAGE (approximately 15 cm x 20 cm, 20 lanes per gel
- $PAP^{35}S$ (prepared in-house using the PAPS Synthesis Kit (Catalog # EA005), ~1 µM = ~2 x 106 cpm/µL)
- Gel loading buffer: 0.15 M Tris, 20.8 mM SDS, 1.15 M Glycine, 174 µM Bromophenol Blue, 30% Glycerol
- Blotting paper (Fisher Scientific, Catalog # 05-714-4)
- Gel dryer
- Glogos® II autorad markers (Stratagene, Catalog # 420202) or equivalent
- Blue sensitive medical X-ray film
- X-ray film cassette
- Film developer (Konica SRX-101A Medical Film Processor) or equivalent
- Liquid scintillation counter (Beckman Coulter, Model # LS5000TD) or equivalent
- Liquid scintillation fluid (Beckman Coulter, Catalog # 141349) or equivalent

Assay

1. Create Radiolabeled Keratan Sulfate Mixture containing 0.1 mg/mL Keratan Sulfate, 12 µg/mL rhCHST-1, and 0.025 µM $PAP^{35}S$ in Labeling Buffer.
2. Incubate Keratan Sulfate Mixture at 37 °C for 1.5 hours.
3. Dilute incubated Keratan Sulfate Mixture 3 fold in Assay Buffer.
4. Dilute rF.k. Endo-β-galactosidase to 1.334 µg/mL in Assay Buffer.
5. Combine 15 µL of 1.334 µg/mL rF.k. Endo-β-galactosidase with 15 µL diluted Keratan Sulfate Mixture. Include a control containing 15 µL Assay Buffer and 15 µL Keratan Sulfate Mixture.
6. Incubate reactions and control at 37 °C for 20 minutes.
7. Add 15 µL gel loading buffer to each reaction and control. Mix.
8. Load 30 µL of each sample per lane on a gel. Leave empty lanes between samples.
9. Run at 200 V for 30 minutes.
10. Transfer gel onto blotting paper and dry with gel dryer for 1 hour or until fully dry.
11. Affix two autorad markers to the blotting paper next to the dried gel.
12. In a darkroom expose dried gel to X-ray film by enclosing overnight in a cassette. Develop the following day.
13. Using the dried gel, begin marking regions to be cut out for scintillation counting.
14. Mark a horizontal line across the top of the entire gel just under the bottom of the wells.
15. Using the developed film as an overlay, mark a second line just below the lower edge of the labeled Keratan Sulfate for each well.
16. Draw a third line just below where the labeled product migrated (ignore any free sulfate, appearing equivalent in all lanes, and migrating the furthest). For the control, identify the empty region where the product would appear.
17. The area between the first two lines contains the labeled starting material. The area between the second two lines contains the compact cleavage product resulting from the reaction.
18. Mark vertical lines distinguishing one lane (reaction condition) from another.
19. Cut each region (two per lane) and place each into a separate liquid scintillation vial. Add 5 mL of liquid scintillation fluid to each vial and count vials for ^{35}S.
20. Calculate % cleavage for each reaction.

Final Assay Conditions

Per Reaction:

- Keratan Sulfate: 0.5 µg
- rF.k. Endo-β-galactosidase: 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.

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BACKGROUND

Keratan sulfate (KS) is a special glycosaminoglycan (GAG) found in cornea, cartilage and bone (1). GAGs are a group of linear polysaccharides with different repeating disaccharide units. Other GAGs include heparin, heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), and hyaluronan (HA). Proteoglycans refer to GAG-protein conjugates. Known KS proteoglycans include lumican, keratocan, mimecan, aggrecan and fibromodulin. They have been identified in numerous epithelial and neural tissues in which KS expression responds to embryonic development, physiological variations, and wound healing (1). KS was first identified in 1939 by Suzuki in extracts of cornea (2), and later by Karl Meyer (3). The basic repeating disaccharide unit within KS is -3Galβ1-4GlcNAcβ1 and are polymerized by B4GALT (4) and B3GNT families of glycosyltransferase (5). KS chains are subsequently sulfated by two specific sulfotransferases, CHST1 and CHST6, at a region close to its non-reducing end (6). KS is linked to core proteins either through O-glycan or N-glycan and corneal KS is mostly N-linked glycan (7). This product was purified from cornea of goat eyes through chemical extraction with 4M guanidine-HCl and further purified with anion-exchange chromatography steps. The identity of KS was confirmed by radioisotope ^{35}S labeling with KS specific sulfotransferases, CHST1 and CHST6, followed by Recombinant *F. keratolyticus* Endo-beta-Galactosidase digestion, which also suggested that KS accounts for majority of the mass of this product.

References:

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