Recombinant Human Hyaluronidase 1/HYAL1

Catalog Number: 7358-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Met1-Trp435, with a C-terminal 6-His tag Accession # Q12794
N-terminal Sequence Analysis	Phe22
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	50-60 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze S-35 labeled hyaluronan. <20 ng of rhHYAL1 is required for the 50% hydrolysis of 10 µg hyaluronan, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, 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 Tris, NaCl and Glycerol. 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 NaOAc, pH 4.5
- Gel Running Buffer: 40 mM Tris, 1 mM EDTA, adjust to pH 8.0 with acetic acid
- Recombinant Human Hyaluronidase 1/HYAL1 (rhHYAL1) (Catalog # 7358-GH)
- Recombinant Mouse Carbohydrate Sulfotransferase 3/CHST3 (rmCHST3) (Catalog # 5356-ST)
- 8% SDS-PAGE (approximately 15 cm x 20 cm, 20 lanes per gel)
- Hyaluronan (Catalog # GLR004), 20 mg/mL
- $PAP^{35}S$ (prepared in-house using the PAPS Synthesis Kit (Catalog # EA005), ~1 µM and ~2 x 10^6 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 Sci., Catalog # 05-714-4)
- Gel dryer
- Glogos® II autorad markers (Stratagene, Cat. # 420202) or equiv.
- Blue sensitive medical X-ray film
- X-ray film cassette
- Film developer (Konica SRX-101A Medical Film Processor) or equiv.
- Liquid scintillation counter (Beckman Coulter, Model # LS5000TD) or equiv.
- Liquid scintillation fluid (Beckman Coulter, Catalog # 141349) or equiv.

Assay

1. Dilute rmCHST3 to 0.3 mg/mL in Labeling Buffer and Hyaluronan to 10 mg/mL in diH_2O.
2. Combine 200 µL Labeling Buffer, 60 µL diH_2O, 60 µL $PAP^{35}S$, 40 µL Hyaluronan, and 40 µL rmCHST3 and incubate mixture at 37 °C for 4 hours.
3. Add 200 µL diH_2O to incubated rxn mixture for a final volume of 600 µL (rxn mix sufficient for ~ 40 rxns).
4. Dilute rhHYAL1 to 15, 4.165, 2.08, 1.04, 0.52, 0.26, 0.13, and 0.00667 µg/mL in Assay Buffer.
5. Combine 15 µL of rhHYAL1 at each dilution with 15 µL rxn mix. Include a control containing 15 µL Assay Buffer and 15 µL rxn mix. Incubate at 37 °C for 20 min.
6. Add 15 µL gel loading buffer to each rxn. Mix.
7. Load 30 µL of each rxn per lane on a gel. Leave empty lanes between samples. Run at 200 V for 35 min.
8. Transfer gel onto blotting paper and dry with gel dryer for 1 hour or until fully dry.
9. Affix two autorad markers to the blotting paper next to the dried gel.
10. In a darkroom expose dried gel to X-ray film by enclosing overnight in a cassette. Develop the film the following day.
11. Using the dried gel, begin marking regions to be cut out for scintillation counting. Mark a horizontal line across the top of the entire gel just under the bottom of the loading wells.
12. Using the developed film as an overlay, mark a second line below the lower edge of the labeled hyaluronan.
13. Draw a third line just below where the labeled product migrated (ignore any free sulfate, appearing equivalent in all lanes, and migrating the furthest). Note: It will be easiest to use the highest enzyme-containing lanes to identify the product. For the control, identify the empty region where the product would appear.
14. The area between the first two lines is considered to contain the labeled starting material. The area between the second two lines is considered to contain the compact cleavage product resulting from the reaction.
15. Mark vertical lines distinguishing one lane (reaction condition) from another.
16. Cut each region (two per lane) and place each into a separate liquid scintillation vial.Add 5 mL liquid scintillation fluid to each vial and count the vials for ^{35}S.
17. Determine the amount of rhHYAL1 required for 50% cleavage by plotting % cleavage vs. ng of rhHYA-L1 with 4-PL fitting.

Final Assay Conditions

- Hyaluronan: 10 µg
- rhHYAL1: 225, 62.5, 31.3, 15.6, 7.8, 3.9, 1.95 and 0.1 ng

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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.

BACKGROUND

Human hyaluronidases (HYALs) are a group of five endo-β-N-acetyl-hexosaminidases that include HYAL1, HYAL2, HYAL3, HYAL4, and SPAM1 (PH20) (1, 2). While HYAL1, HYAL2 and SPAM1 are endo-β-N-acetyl-glucosaminidase that are mainly active on hyaluronan with little activity on chondroitin sulfate (3, 4, 5), HYAL4 is mainly active on chondroitin sulfate (type C and D in particular) (6). Hyaluronan and chondroitin sulfate are abundant extracellular matrix components that have numerous biological functions (7). The backbone of chondroitin sulfate composed of repeating units of -4GlcAβ1-3GalNAcβ1- most closely resembles to that of hyaluronan composed of repeating units of -4GlcAβ1-3GlcNAcβ1-, which may partially explain the overlapping substrate specificity of most hyaluronidases. HYAL1 is a lysosomal hyaluronidase with optimal activity around pH 4.0 (8). It is highly expressed in the liver, kidney and heart, and is the predominant hyaluronidase found in plasma (9). Defects in HYAL1 are associated with mucopolysaccharidosis type IX, or hyaluronidase deficiency (10). Surprisingly, HYAL1 has been reported both as a tumor promoter and suppressor (11, 12).The enzymatic activity of recombinant human HYAL1 was measured using ^{35}S-labeled hyaluronan as substrate.

References:

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