

Fluoresceinamine -labeled Sodium Heparin (N1)

Product code: AMS.CSR-FAHEP-N1

Volume: 3 mL (1 mg/mL, in phosphate buffered saline (PBS) (-))

Appearance: yellow green solution

Source of sodium heparin: Porcine intestine

CAS number of sodium heparin: 9041-08-1

Fluorescent probe: Fluoresceinamine

CAS number of fluorescent probe: 3326-34-9

Outline: Heparin (Hep) is a sulfated glycosaminoglycan composed of repeating disaccharide units of D-iduronic acid (IdoUA) or D-glucuronic acid (GlcUA) and N-acetyl-D-glucosamine or N-sulfo-glucosamine. Hep is abundant in mast cells of intestine or lung exists as unbranched polysaccharide chains. This product is prepared by the fluorescent labeling of Hep derived from porcine intestine according to the method of Ogamo et al.¹⁾. Fluoresceinamine molecules are chemically attached to carboxyl groups of the GlcUA or IdoUA of Hep. This solution is dissolved in PBS (-) and sterilized by filtration. The endotoxin content is in accordance with the product specifications. The excitation wavelength is 490~500 nm and the emission wavelength is 515~525 nm. The enclosed Certification of Analysis lists actual values for product specifications.

Handling precautions:

- 1) Protect from light as much as possible. Product can be used at room temperature when protected from strong light.
- 2) After thawing, gently agitate the vial before use.
- 3) Store protected from light at -20°C or below. We recommend storing in aliquots appropriate for anticipated usage.
- 4) Fluorescence intensity varies with pH of the solution and is lower under acidic conditions. Note the pH of the sample solution when measuring fluorescence intensity.
- 5) This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Reference:

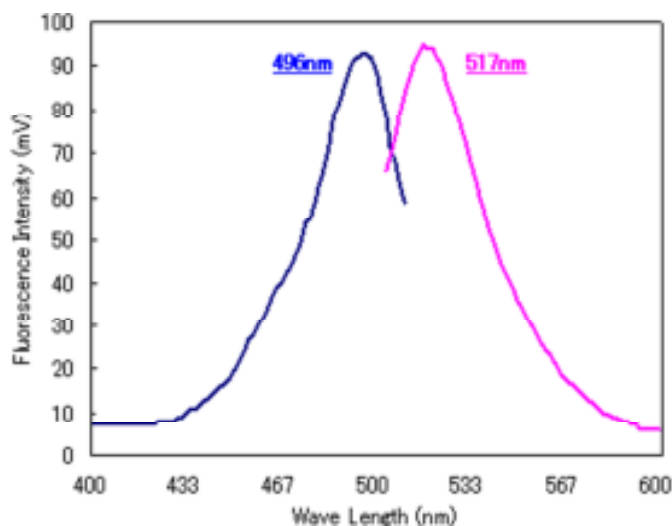
- 1) Ogamo A et al.: Carbohydr. Res., **105**, 69 (1982)

Specifications

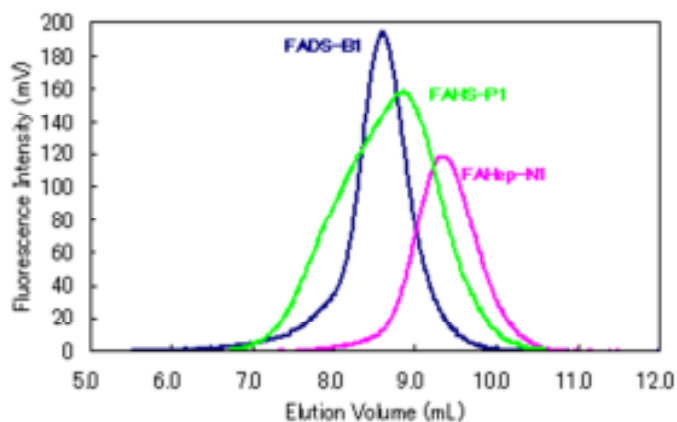
Appearance	yellow green solution
Concentration of FADS	0.90 ~ 1.10mg/mL
Wavelength of Excitation	490 ~ 500 nm
Wavelength of Emission	515 ~ 525 nm
Degree of Substitution*	0.600 ~ 1.100%
Fluorescent Purity on Electrophoresis	one band at same mobility with original NaHep
Concentration of Endotoxin	< 0.25 EU/mL
Storage	Store protected from light at -20°C or below. We recommend storing in aliquots appropriate for anticipated usage.

NaHep: Sodium Heparin

*Expressed as the molar percentage of bound fluoresceinamine groups to disaccharide units.



Excitation and emission spectrum of FAHep-N1 (pH10)



Elution profiles of FADS-B1, FAHep-N1 and FAHS-P1 on GPC
(Column: TSKgel G4000PWXL (7.8mmI.D. × 30cm × 1))

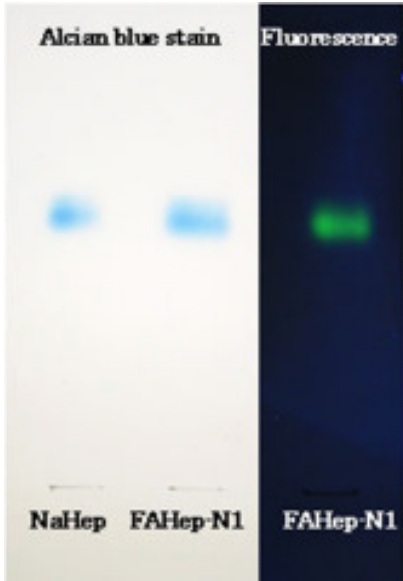
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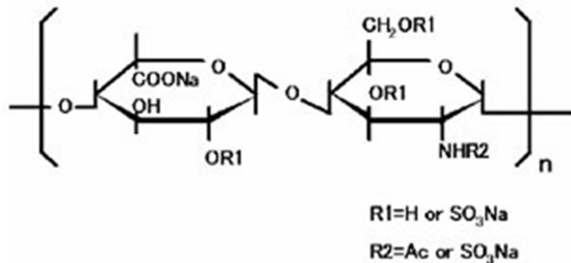
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Cellulose acetate membrane electrophoresis of FAHep-N1
NaHep: Sodium Heparin

Raw materials of Fluorescently labelled Glycosaminoglycans

Product Name	Raw Material	Product Name	Raw Material
AMS.CSR-FAHA-H1, H2	Streptococcus sp.	AMS.CSR-FACS-A1	whale cartilage
AMS.CSR-FAHA-M1, M2	chicken comb	AMS.CSR-FACS-C1	shark cartilage
AMS.CSR-FAHA-L1, L2	Streptococcus sp.	AMS.CSR-FACS-D1	shark cartilage
AMS.CSR-FAHA-S1	Streptococcus sp.	AMS.CSR-FACS-E1	squid cartilage
AMS.CSR-FAHA-T1	Streptococcus sp.	AMS.CSR-FADS-B1	porcine skin
AMS.CSR-FAHep-N1	porcine intestine	AMS.CSR-FAHS-P1	porcine kidney



Typical disaccharide formula of Sodium Heparin

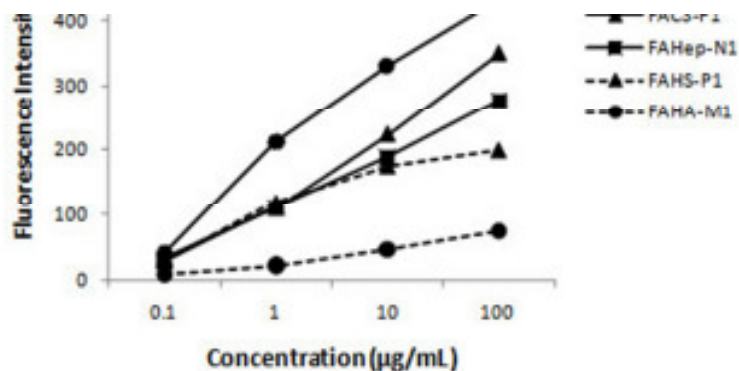
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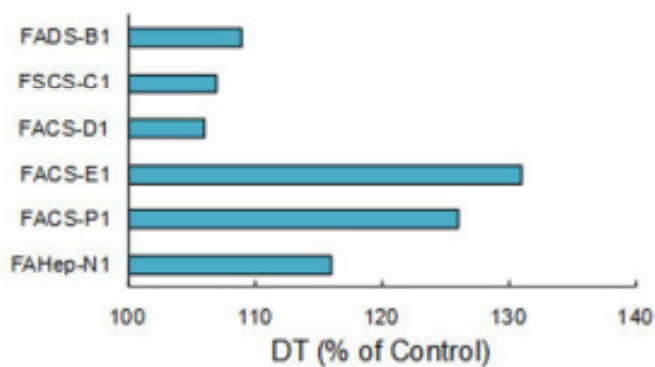
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Analysis of binding activities with bFGF

Basic fibroblast growth factor (bFGF, 10µg/mL in PBS, 100 µL/well) was added into 96well plate (Nunc, 437111) and incubated at 37°C for 1h. The plate was washed with PBS, and then fluorescein-labeled GAG solution with various concentrations in PBS was added to each well (100µL/well) and incubated at 37°C for 1h. After washing the plate, 0.1N NaOH was added (100µL/well), and the fluorescence intensity was measured using a fluoro-plate reader with an excitation wavelength of 485 nm and an emission wavelength of 535 nm. It was observed that the fluorescence intensity was increased with the concentration of FACS-E1, FAHS-P1 FAHep-N1 and FAHS-P1 whereas the intensity was little increased in the case of FAHA-M1. The result suggests an interaction between sulfated GAG and bFGF.



Analysis of binding activities with BMP-4 by fluorescence correlation spectroscopy (FCS)

Fluorescence correlation spectroscopy (FCS) is an analytical method of molecular-association or dissociation with one molecular level. The interaction of molecules can be detected as a prolongation of average diffusion time (DT) derived from a fluorescent molecule. The figure shows the effect of recombinant human bone morphogenetic protein-4 (rhBMP-4) on the DT of various fluoresceinamine-labeled GAGs. The result indicates relatively strong interactions between rhBMP-4 and FACS-E1, FACS-P1 or FAHep-N1. (J Cell Physiol, 2008, 217(3):769-777)