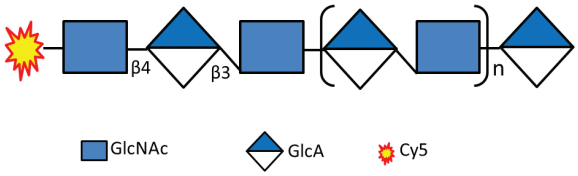
Cy5 Labeled Hyaluronan (Low MW)

Catalog Number: GL401
Lot Number: DOZK0124031
Size: 200 pmol



n, number of repeating disaccharide units, range about from 5 to 100

DESCRIPTION

Formulation	Supplied in 25 mM Tris, 150 mM NaCl, pH 7.5
Storage & Stability	Store at < -20 °C. Good for 6 months from date of receipt.

APPLICATIONS

- Studying hyaluronan protein binding.
- Used as a substrate for various hyaluronan specific hydrolases and lyases.

KEY FEATURES & BENEFITS

- Excitation at 649 nm and emission at 671 nm.
- The fluorescent dye Cy5 is conjugated to the non-reducing end GlcNAc residue through enzymatic conjugation.
- Can be separated on 15-17% SDS-PAGE and directly visualized as a ladder containing 5-100 repeating units (RU) of the HA disaccharide GlcA-β,3-GlcNAc.
- Linear response range for Cy5 labeled glycans can be from 10 fmol to 100 pmol, depending on the sensitivity of detection.

RELATED REAGENTS

Click Chemistry

- GDP-Cy5-Fucose (ES301)
- CMP-Cy5-Sialic Acid (ES302)
- GDP-Cy3-Fucose (ES401)
- CMP-Cy3-Sialic Acid (ES402)

Enzymes and Detection Reagents

- Various Hyaluronidases and Hyaluronan Lyase
- HA-binding Proteins
- Hyaluronan

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SAMPLE ASSAY PROTOCOL

For using Cy5 Labeled Hyaluronan (Low MW)/Cy5-LMW HA as substrate for Recombinant Human HYAL1 Assay.
Protocols are guidelines. Parameters need to be optimized by end users. Cy5-LMW HA contains multiple bands and it is suggested to have 2 pmol for each HYAL1 digestion.

OTHER MATERIALS REQUIRED

- Assay Buffer: 50 mM NaOAc, pH 4.0
- Recombinant Human Hyaluronidase 1/HYAL1 Protein, CF (rhHYAL1) (R&D Systems®, Catalog # 7358-GH)
- 15% SDS-PAGE
- 6X Gel Loading Dye
- A fluorescent imager

FINAL ASSAY CONDITIONS PER REACTION

- rhHYAL1: 0.08-10 ng
- Cy5-LMW HA: 2 pmol

ASSAY PROCEDURE

1. Dilute rhHYAL1 to 1 ng/µL in Assay Buffer.
2. Complete eight 2-fold serial dilutions using 10 µL of the diluted rhHYAL1 (10 ng). This has a range of 0.08-10 ng.
3. Dilute Cy5-LMW HA to 0.2 µM in Assay Buffer.
4. Add 10 µL of the Cy5-LMW HA to each dilution.
5. Prepare a negative control by mixing 10 µL of the Cy5-LMW HA with 10 µL of Assay Buffer.
6. Incubate the reaction and control at 37 °C for 20 minutes.
7. Stop the reactions and controls by adding 5 µL of 6X gel loading dye to each of the above tubes.
8. Load 12.5 µL each of the above samples per well on an SDS-PAGE and run at 10 volts/cm until the dye front is more than two thirds of the gel.
9. Visualize the gel with a fluorescent imager for 10 seconds.