

Product Data Sheet

Product Name: FITC-HA (MW 4000000)

Cat. No.: GC80377

Chemical Properties

Cas. No.

Formula

M.Wt 4000000

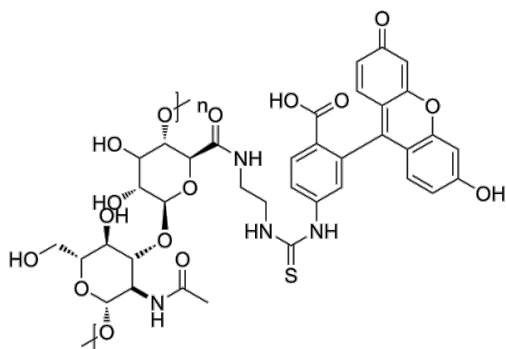
Solubility

Storage Store at -20°C, protect from light

General For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the tips ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Evaluation sample solution : ship with blue ice All other available size: ship with RT, or Condition blue ice upon request.

Structure



Background

FITC -HA (MW 4000000) is hyaluronic acid (HA) labeled with FITC. FITC -HA retains the ability of HA to bind to receptors (such as CD44) and form extracellular matrices, while it can be detected by fluorescence microscopy or flow cytometry for tracing the localization, binding, internalization and metabolic pathways of HA in cells, tissues or living organisms (Ex/Em $\approx$ 490/520 nM) [1] [2].

In Vitro, FITC-HA exhibits strong penetration and is prone to endocytosis at low molecular weights (< 50 kDa), making it suitable for skin/mucosal penetration studies; at high molecular weights (> 100 kDa), it shows higher affinity for CD44, is resistant to endocytosis, and has strong retention

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

properties, remaining mainly on the skin surface or in the superficial dermis, thus ideal for local retention research. Guide (The following is our recommended protocol, which serves as a guide only and should be modified according to your specific needs).

1. Sample and Cell Preparation Cell models: Commonly used are articular chondrocytes, COS-7 cells (for transfection studies), or skin-related cells. Cells are usually pre-seeded or cultured in a suitable carrier. Removal of endogenous HA (optional but critical): To expose all CD44 binding sites and achieve a clear background, cells are typically treated with streptomyces hyaluronidase (e.g., 2 U/mL in serum-containing medium, incubated overnight at 37°C) to remove pre-existing HA matrix on the cell surface. Probe preparation: Dilute the FITC-HA stock solution to working concentration with an appropriate buffer (e.g., PBS) or culture medium. Depending on the experimental purpose, FITC-HA can be used alone, or pre-incubated with unlabeled HA, proteoglycans (e.g., aggrecan monomers, HABP), or other molecules to be studied to form complexes prior to use.

2. Incubation and Treatment

2.1 Binding assay (studying cell surface binding): Incubate cells with FITC-HA working solution at 4°C for 1 hour. Low temperature inhibits internalization, allowing fluorescent signals to remain mainly on the cell surface. After incubation, wash cells several times with pre-chilled buffer to remove unbound FITC-HA.

2.2 Internalization assay (studying cellular uptake): Incubate cells with FITC-HA working solution at 37°C for several hours. 37°C permits normal endocytic processes to occur. Critical step: Membrane surface fluorescence quenching. To distinguish between cell surface-bound and internalized FITC-HA, trypsin (e.g., 0.25%, treated at 37°C for 30 minutes) is commonly used after incubation to digest and remove probe bound to the cell surface. Only FITC-HA internalized into cells resists this treatment and is retained.

3. Detection and Analysis

3.1 Fixation and permeabilization: Cells are fixed with 1% paraformaldehyde and then permeabilized with 0.1% Triton X-100 (for observing intracellular signals).

3.2 Imaging and quantification: Confocal fluorescence microscopy is the primary observation tool. Z-axis sectional scanning enables three-dimensional distribution imaging of FITC-HA in skin or within cells. Fluorescence intensity at specific depths (e.g., stratum corneum, epidermis/dermis) can be calculated for semi-quantitation. Flow cytometry: Used for quantitative analysis of the mean fluorescence intensity of FITC-HA binding or internalization in cell populations. Co-localization analysis: Co-staining can be performed with organelle-specific dyes for lysosomes, endosomes, etc. (e.g., LysoTracker Red), and subcellular localization of FITC-HA is confirmed by fluorescence overlay.

4. Specificity Controls Key control experiments should be set up to verify the mechanism: HA-dependent control: Addition of hyaluronidase to the incubation system abolishes FITC-HA binding/internalization, confirming dependence on intact HA chains. Receptor-dependent control: Gain-of-function: Transfection of wild-type CD44 into CD44-negative cells (e.g., COS-7) should confer the ability to bind/internalize FITC-HA. Loss-of-function: Transfection of dominant-negative

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

CD44 mutants into CD44-positive cells (e.g., chondrocytes) should suppress their ability to bind/internalize FITC-HA. Competitive inhibition: Use of excess unlabeled HA to compete with FITC-HA should inhibit FITC-HA signals in a dose-dependent manner.

References:

- [1]. Embry JJ, et al. G1 domain of aggrecan cointernalizes with hyaluronan via a CD44-mediated mechanism in bovine articular chondrocytes. *Arthritis Rheum.* 2003;48(12):3431-3441.
- [2]. Kim KT, et al. Novel reverse electrodialysis-driven iontophoretic system for topical and transdermal delivery of poorly permeable therapeutic agents. *Drug Deliv.* 2017;24(1):1204-1215.

Caution: Product has not been fully validated for medical applications. For research use only.

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA