V. 2.0

Fibronectin (HiLyte Fluor™ 488 labeled)
Source: Bovine plasma
Cat. # FNR02

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Background Information

The Extracellular Matrix (ECM) is composed of collagen, non-collagenous glycoproteins and proteoglycans. These components are secreted from cells to create an ECM meshwork that surrounds cells and tissues. The ECM regulates many aspects of cellular function, including the cells dynamic behavior , cytoskeletal organization and intercellular comminucation (1).

Fibronectin is a high-molecular weight (~440kDa) glycoprotein found in the extracellular matrix and in blood plasma. It is made up of two subunits that vary in size between 235-270 kDa (due to alternate splicing). The secreted fibronectin dimer is a soluble protein which polymerizes to higher order fibrils in the ECM.

Fibronectin plays a major role in cell adhesion, growth, migration , actin dynamics and differentiation, and it is important for processes such as wound healing and embryonic development (2). Many of these functions are mediated through fibronectin binding to integrin receptor proteins (2). Altered fibronectin expression, degradation, and organization has been associated with a number of pathologies, including cancer and fibrosis (3).

In addition to integrins, fibronectin also binds extracellular matrix components such as collagen, fibrin and heparan sulfate proteoglycans (e.g. syndecans).

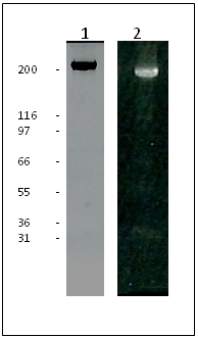
Material

Fibronectin is purified from bovine plasma. Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gel. HiLyte Fluor™ 488 labeled fibronectin is >80% pure (Figure 1).

The protein is modified to contain covalently linked HiLyte Fluor™ 488 at random surface lysines (4). An activated ester of the fluorochrome is used to label the protein. Labeling stoichiometry is determined by spectroscopic measurement of protein and dye concentrations. Final labeling stoichiometry is 1-3 dyes per protein molecule (Figure 2). No free dye is apparent in the final product (Figure 1). HiLyte Fluor™ 488 labeled fibronectin can be detected using a filter set of 502 nm excitation and 527 nm emission.

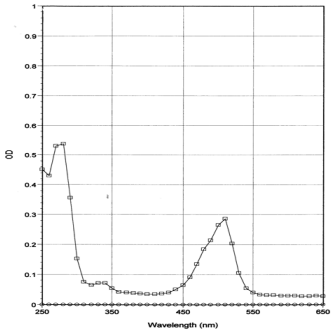
Fibronectin runs as individual subunits on SDS-PAGE with an apparent molecular weight of 230 kDa. FNR02 is supplied as an orange lyophilized powder. Each vial of FNR02 contains 20 µg protein.

Figure 1: HiLyte Fluor™ 488 labeled Fibronectin Purity Determination



Legend: 20 µg of unlabeled fibronection (Lane 1) and 20 µg of HiLyte Fluor™ 488 labeled fibronectin (Lane 2) was separated by electrophoresis in a 4-20% SDS-PAGE system. The unlabeled protein was stained with Coomassie Blue and visualized in white light. The HiLyte Fluor™ 488 labeled protein was visualized under UV light, no free dye was observed in the dye front. Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Figure 2: Absorption scan of HiLyte Fluor™ 488 labeled fibronectin in solution



Legend: FNR02 was diluted with Milli-Q water and its absorbance spectrum was scanned between 250 and 650 nm. HiLyte Fluor™ 488 labeling stoichiometry was calculated to be 1-3 dyes per fibronectin protein using the absorbancy maximum for HiLyte 488™ at 527 nm and the Beer-Lambert law. Dye extinction coefficient when protein bound is 70,000$M^{-1}cm^{-1}$

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized protein can be stored desiccated to <10% humidity at 4°C for 6 months in the dark. For reconstitution, briefly centrifuge to collect the product at the bottom of the tube and resuspend to 1 mg/ml with 20 µl cold distilled water. The protein will then be in the following buffer: 20mM Tris-

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HCl pH 7.6, 20 mM NaCl, 0.1 mM EDTA, 15 mM BME, and 5% (w/v) sucrose. Avoid excessive mixing as this can cause protein aggregation. The concentrated protein should be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at –70°C where it is stable for 6 months. For working concentrations, further dilution of the HiLyte Fluor™ 488 labeled fibronectin should be made in a suitable buffer or tissue culture media. HiLyte Fluor™ 488 labeled fibronectin is a labile protein and should be handled with care. Avoid repeated freeze-thaw cycles.

Biological Activity Assay

Proteolytic degradation of the ECM is a critical step during cell invasion and is necessary for both physiological and pathological processes. HiLyte Fluor™ 488 labeled fibronectin can be used as an ECM substrate to monitor invasion through observation of ECM degradation (5).

Product Uses

- Observation of fibronectin matrix assembly and cell adhesion
- Cell invasion assays (5)
- FACS analysis of fibronectin binding cells.

References

1. Guidebook to the extracellular matrix and adhesion proteins. 1993. Oxford University Press. Ed. Kreis T and Vale R.
2. Pankov R, Yamada KM . 2002. "Fibronectin at a glance". *Journal of Cell Sci.* 20 **115**: 3861-3863.
3. Williams CM, Engler AJ, Slone RD, Galante LL, Schwarzbauer JE. 2008. “Fibronectin expression modulates mammary epithelial cell proliferation during acinar differentiation. *Cancer Research.* 9 **68**: 3185-8192.
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5. Artym VV. Et al. 2009. ECM degradation assay for analyzing local cell invasion. *Methods in molecular biology, Extracellular matrix protocols*, vol. **522**: 211-219.Humana Press.

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