Far-red HiLyte Fluor™ 647 Labeled Fluorescent Fibronectin V. 1.2
Source: Bovine plasma
Cat. # FNR04
Lot # Amount:

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 communication (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. Far-red fluorescent fibronectin is >80% pure (Figure 1).

The protein is modified to contain covalently linked Far-red fluorescent HiLyte Fluor™ 647 at random surface lysines. An activated ester of HiLyte Fluor™ 647 [succinimidyl ester] 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). Far-red fluorescent fibronectin can be detected using a filter set of 640nm excitation and 670nm emission.

Fibronectin runs as individual subunits on SDS-PAGE with an apparent molecular weight of 230 kDa. FNR04 is supplied as a blue lyophilized powder. Each vial of FNR04 contains 20 µg protein.

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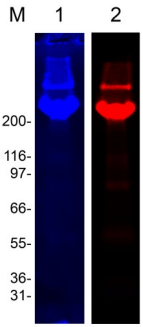
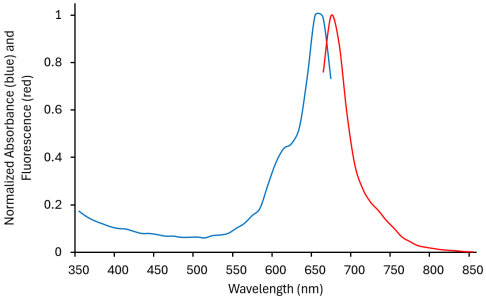
Figure 1: Far-red fluorescent fibronectin Purity Determination



Legend: 20 µg of FNR04 was analyzed by electrophoresis in a 4-20% SDS-PAGE system. A Licor Odessy gel analysis was performed at 700nm (Coomassie, lane 1), and 680nm (HiLyte647, lane 2). The Far-red fluorescent labeled protein was visualized at 240 kDal and 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: Absorbance and fluorescence scan of Far-red fluorescent fibronectin in solution



Legend: FNR04 was diluted with nanopure water and its absorbance (blue line) and fluorescence (red line, with emission 640 nm) spectra were scanned between 350 and 850 nm. Far-red fluorescent labeling stoichiometry was calculated to be 1-3 dyes per fibronectin protein using the absorbance maximum for Far-red fluorescent at 653 nm and the Beer-Lambert law. The extinction coefficient of the dye is 250,000 $M^{-1}cm^{-1}$.

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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 room temperature sterile nanopure water. Let the protein re-dissolve for 1-2 minutes without mixing; after 1-2 minutes the protein solution can be gently pipetted up and down 2-3 times to ensure complete resuspension. Excessive mixing should be avoided as this can cause protein aggregation. Once product is resuspended, place on ice. The protein will be in the following buffer: 20mM Tris-HCl pH 7.6, 20 mM NaCl, 0.1 mM EDTA, 15 mM BME, and 5% (w/v) sucrose. 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 Far-red fluorescent fibronectin should be made in a suitable buffer or tissue culture media. Far-red fluorescent 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. Far-red fluorescent fibronectin can be used as an ECM substrate to monitor invasion through observation of ECM degradation (4).

Product Uses

- Observation of fibronectin matrix assembly and cell adhesion
- Cell invasion assays (4)
- 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.
4. 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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