

Biotin Labeled Fibronectin

Source: Bovine plasma

Cat. # FNR03

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 cell's dynamic behavior, cytoskeletal organization and intercellular communication (1).

Fibronectin is a high-molecular weight (~440 kDa) 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).

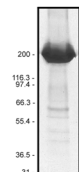
Material

Fibronectin is purified from bovine plasma. Protein purity is determined by scanning densitometry of coomassie blue stained protein on a 4-20% polyacrylamide gel. Biotinylated fibronectin is >80% pure (Figure 1).

The protein is modified to contain covalently linked biotins at random surface lysines. A long-chain activated ester of biotin [biotin-XX, succinimidyl ester] is used to label the protein. Labeling efficiency is determined by the ability to detect 10 ng biotinylated fibronectin using alkaline phosphatase conjugated streptavidin (Figure 2).

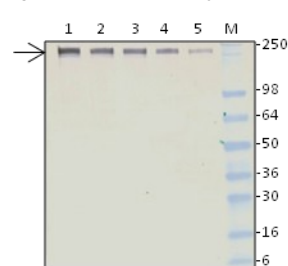
Fibronectin runs as individual subunits on SDS-PAGE with an apparent molecular weight of 230 kDa. FNR03 is supplied as a white lyophilized powder. Each vial contains 20 µg protein.

Figure 1: Fibronectin Purity Determination



Legend: 50 µg of biotin fibronectin was separated by electrophoresis in a 4-20% SDS-PAGE system. The protein was stained with coomassie blue. Protein quantitation was determined with the Precision Red™ Protein Assay Reagent (Cat. # ADV02). Mark12 molecular weight markers are from Invitrogen.

Figure 2: Detection of Biotinylated Fibronectin.



Legend: Serial dilutions of biotinylated fibronectin were separated by electrophoresis on a 4-20% polyacrylamide gel, blotted to PVDF, probed with a 1:1000 dilution of streptavidin alkaline phosphatase (Sigma) and detected with 1-Step NBT/BCIP reagent™ (Pierce). Lane 1, 100 ng, Lane 2, 50 ng, Lane 3, 40 ng, Lane 4, 20 ng, and Lane 5, 10 ng of biotinylated fibronectin. Lane M, SeeBlue™ molecular weight markers (Invitrogen). Arrow indicates biotinylated fibronectin.

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 distilled 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: 20 mM 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 biotin fibronectin should be made in a suitable buffer or tissue culture media. Biotin fibronectin is a labile protein and should be handled with care. Avoid repeated freeze-thaw cycles.

Biological Activity Assay

Biological activity of biotinylated fibronectin can be determined by the ability of the soluble reagent to incorporate into extracellular fibrillar matrices. This assay is a non-radioactive alternative to quantitation of matrix assembly in response to various signaling events (4).

Product Uses

- Observation of fibronectin matrix assembly (4)
- Cell adhesion assays
- Bioengineering; study of cell adhesion to polymers (5)

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. Pankov R and Yamada KM. 2004. Non-radioactive quantification of fibronectin matrix assembly. *Curr. Protocols in Cell Biol.* **S25**, 10.13.1—10.13.9.
5. Lehnert M, et al. 2011. Adsorption and conformation behavior of biotinylated fibronectin on streptavidin-modified TiO(X) surfaces studied by SPR and AFM. *Langmuir*, **27**, 7743-7751.

Product Citations/Related Products

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