Human Plasma Fibronectin

#Cat: NB-26-01731 Size:1mg

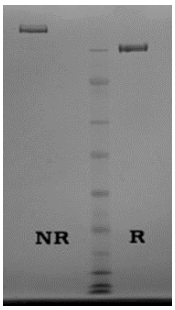
GENERAL INFORMATION

Fibronectin (cold-insoluble globulin) is a high molecular weight, adhesive, glycoprotein found in both plasma and the extracellular matrix. Fibronectin is composed of two peptide chains of approximately 275,000 molecular weight which are linked through two interchain disulfide bonds at the COOH-terminal end of the molecule. The structure of fibronectin is characterized by three different types of repeating homologous sequence units. The 45 amino acid type- I repeat constitutes the NH2- and COOH-terminal ends of the protein. The two 60 amino acid type-II segments follow the first nine type-I repeats at the NH2-terminus. The 90 amino acid type-III segments occupy the central region of the fibronectin molecule. Structural differences between plasma and cellular fibronectin as well as between the two subunits of plasma fibronectin have been identified. These differences likely originate due to transcriptional and posttranscriptional events involving mRNA splicing.

The apparent function of fibronectin is to mediate cell attachment by interacting with cell surface receptors and extracellular matrix components. Fibronectin contains an Arg-Gly-Asp-Ser (RGDS) cell attachment-promoting sequence within one of the type-III homology segments in the middle of the molecule. This RGDS site is recognized by specific RGDS-dependent cell receptors which are members of the integrin family of proteins. Fibronectin binding to activated platelets is mediated through the RGDS cell adhesion sequence. Other binding domains specific for such extracellular macromolecules as heparin, fibrin(ogen), and collagen have been identified and may be important in the fibronectin-mediated adhesion of platelets to the extracellular matrix of endothelial cells. Fibrin-fibronectin complexes are stabilized by the factor XIIIa-catalyzed covalent cross-linking of fibronectin to a chain of fibrin.

Fibronectin is isolated from human plasma by thermal precipitation, ion exchange, and gelatin-agarose affinity chromatography. The purified protein is supplied as a lyophilizate. Purity is assessed by SDS-PAGE analysis.

Gel	Novex 4-12% Bis-Tris
Load	Load: Human Fibronectin, 1 µg per lane
Buffer	MOPS
Standard	SeeBluePlus 2; Myosin (191 kDa), Phosphorylase B (97 kDa), BSA (64 kDa), Glutamic Dehydrogenase (51 kDa), Alcohol Dehydrogenase (39 kDa), Carbonic Anhydrase (28 kDa), Myoglobin Red (19 kDa), Lysozyme (14 kDa)



*Donors tested negative for HBsAg, HCV, HIV1 and HIV2 antibodies.

Localization	Plasma, 0.3 mg/ml extracellular matrix
Mode of action	mediates cell attachment to extracellular matrix
Molecular weight	550,000
Extinction coefficient	E 1 % 1 c m, 280 nm=
Isoelectric point	~5.0
Structure	Heterodimer
Percent carbohydrate	4-10%, depending on source
Concentration	1 mg/ml
Storage Buffer	0.15 M NaCl, 50 mM Tris, pH 7.5

QUALITY CONTROL

Fibronectin quality was assured with a 0.15mM NaCl, 50 mM Tris chloride, pH 7.5, fibronectin 1 mg/ml. Sterilized by
0.22 um membrane filtration. Under reducing conditions, fibronectin appeared as a doublet of 230 and 220 kDa. ELISA assay showed that absorbance was directly proportional to the logarithm of fibronectin concentration. Cell adhesion assays indicated that a coating with as low as 0.1 µg/cm2 of fibronectin significantly promoted endothelial cell adhesion compared with non-coated controls.

STORAGE/HANDLING

It is recommended to store the product as single use aliquots at -80˚C. Thawing should be done slowly at 37˚C with no agitation. Material that fails to dissolve can be removed by centrifugation. Avoid repeated freeze/thaw cycles.

APPLICATION

Recommended for use as a cell culture substratum at 1-5 µg/cm2. Optimal concentration depends on cell type.

COATING INSTRUCTIONS

1. Dilute fibronectin in Ca2+, Mg2+-free phosphate buffered saline. Coat the culture surface at 1-5 µg/cm2 with a minimal volume.

2. Incubate at 37˚C incubator or at room temperature for at least 2 hours. Aspirate remaining fibronectin solution and rinse with DI H2O. The culture vessels are now ready to use.