

Certificate of Analysis

Product: Affinity Purified Anti-Fibronectin (Human Plasma) [Rabbit] Minimum Cross Reactivity to collagens and non-collagen extracellular matrix proteins.

Code: 600-401-117-0.5

Lot # 17651

Size: 500 µg

Antibody Concentration: 1.0 mg/ml (by A₂₈₀ nm)

Buffer: 0.125M Sodium Borate, 0.075M Sodium Chloride, 0.005M EDTA; pH 8.0 **Physical State:** Liquid (sterile filtered)

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Application Note(s): Anti-Fibronectin antibodies have been used for indirect trapping ELISA for quantitation of antigen in serum using a standard curve, for immunoprecipitation and for western blotting for highly sensitive qualitative analysis. Rockland's anti-Fibronectin detects intact fibronectin (Invitrogen, Cat. No. 33016-015) by western blot after digestion by Matrix Metalloproteinase-3 (MMP-3) overnight at 37° C. Separation was performed using a 4-12% Tris-Glycine gel. Under these conditions a sizeable, dark band at ~220 kDa representing the undigested fibronectin, as well as many, smaller bands representing the variably sized fragments resulting from fibronectin digestion by MMP-3 were noted. For immunohisto-chemistry paraffin embedded tissue preparation is recommended.

Background Information: Human fibronectin has a molecular weight of 450,000 daltons when purified in an intact form from plasma. Fibronectin is a glycoprotein synthesized in the liver for the circulating blood plasma form, and is synthesized by many mesenchymal cells, for the extracellular matrix form. It is composed of two similar, but not identical protein chains, which are linked by two disulfide linkages at the C-terminal end of the chains. The chains are composed of domains which have specific secondary structures linked together by regions which are especially susceptible to proteolytic action. For this reason, detection by immunoblot (western) may show considerable variability in the observed apparent molecular weights, which is predicated on the source of the fibronectin, and to what degree proteolysis has occurred. Bands approximately 225 kDa should be observed after SDS-PAGE when reducing and denaturing conditions are used.

Recommended Dilution(s):	ELISA	1:4,000 - 1:8,000
	WESTERN BLOT	1:4,000 - 1:8,000
	IMMUNOHISTOCHEMISTRY	1:50 - 1:200
	OTHER APPLICATIONS	User Optimized

Storage Conditions: Store vial at 4° C prior to opening. This product is stable at 4° C as an undiluted liquid. Dilute only prior to immediate use. For extended storage mix with an equal volume of glycerol, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening product.

Purity and Specificity: This product has been prepared by immunoaffinity chromatography using immobilized antigens followed by extensive cross-adsorption against human serum proteins and collagen and non-collagen extracellular matrix proteins to remove any unwanted specificities. Typically less than 1% cross reactivity against other extracellular matrix proteins was detected by ELISA against purified standards. This antibody reacts with human Fibronectin and has negligible cross-reactivity with Type I, II, III, IV, V or VI Collagens or Laminin. Non-specific cross reaction of anti-Fibronectin antibodies with other human serum proteins or non-Fibronectin extracellular matrix proteins is negligible.

Immunogen: Fibronectin was purified from Human plasma by binding to a denatured gelatin column followed by elution with high concentrations of arginine. The eluted material was further purified by gel filtration. Immunization occurred after single-band purity was assessed by SDS-PAGE.

Background Reference: Yamada, K.M., (1983) *Annu. Rev. Biochem.*, **52**:761.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.