Product Specification Sheet

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

Code: 600-401-117-0.1 **Lot #** 12305 **Size:** 100 μg

Physical State: Liquid (sterile filtered) **Antibody Concentration:** 1.0 mg/ml (by A_{280} nm)

Buffer: 0.125M Sodium Borate, 0.075M Sodium Chloride, 0.005M EDTA; pH 8.0

Stabilizer: None **Preservative:** None

Application(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.

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): This product was assayed by immunoblot and found to be reactive against Fibronectin at a dilution of 1:5,000 to 1:10,000. Rockland's anti-Fibronectin detects by western blot intact fibronectin (Invitrogen, Cat. No. 33016-015) 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. The blots were developed using the colorimetric Alkaline Phosphatase method. This product was also assayed against 1.0 ug of Fibronectin in a standard sandwich ELISA using Peroxidase conjugated Affinity Purified anti-Rabbit IgG [H&L] (Goat) code #611-1302 and (ABTS (2,2'-azino-bis-[3-ethylbenthiozoline-6-sulfonic acid]) code # ABTS-100 as a substrate for 30 minutes at room temperature. A working dilution of 1:4,000 to 1:8,000 of the stock concentration is suggested for this product. For immunohistochemistry on paraffin embedded tissue dilute the product 1:50 to 1:200. Optimal titers for other applications should be determined by the researcher.

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.

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.