

**Product No. F-3648**  
**Lot 047H4837**

**Anti-Human Fibronectin**  
Developed in Rabbit  
Affinity Isolated Antibody

Antiserum is developed in rabbit using purified human fibronectin as the immunogen. Affinity isolated antigen specific antibody is obtained from rabbit anti-human fibronectin antiserum by immunospecific purification which removes essentially all rabbit serum proteins, including immunoglobulins, which do not specifically bind to human fibronectin. The product is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4 containing 1% bovine serum albumin and 0.1% sodium azide (see MSDS)\* as a preservative.

### Specificity

The antiserum is determined to be immunospecific for human fibronectin by immunofluorescent labeling of human fibroblast cell cultures, ELISA and immunoblotting. The product shows no reaction with Collagen IV, laminin, vitronectin and chondroitin sulfate type A, B and C, using dot blot immunoassay. The product cross-reacts with rat, mouse, goat, sheep, guinea pig, bovine, pig and chicken fibronectin. In immunoblotting, a specific band of fibronectin at 220 kD is observed (another band at 94 kD may be also be present) using human plasma and human fibronectin.

The antibody, when used in immunoelectrophoresis, shows 1-2 arcs of precipitation versus normal human plasma.

### Identity and Purity

Identity and purity of the antibody is established by immunoelectrophoresis (IEP). Electrophoresis of the antibody preparation followed by diffusion versus anti-rabbit IgG and anti-rabbit whole serum results in single arcs of precipitation.

### Description

Fibronectin (FN) is a multifunctional, extracellular matrix glycoprotein composed of two nearly identical disulfide-bound polypeptides of molecular weight 220 kD. Cellular fibronectin is structurally and

antigenically similar to cold insoluble globulin from plasma, therefore polyclonal antibodies to either form usually crossreact. Careful analysis of the fibronectin molecule indicate that it contains several functionally and structurally distinct domains which may bind to cell surfaces, collagen, fibrinogen or fibrin, complement, glycosaminoglycans, proteoglycans and heparin. Numerous studies have shown that fibronectin may enhance cell adhesion and spreading and affect the routes of cell migration both *in vivo* and in culture. Moreover, it has been shown that upon malignant transformation many cells lose most of their surface bound fibronectin. Fibronectin has been shown to also play a role in cellular morphology, cytoskeletal organization, phagocytosis, hemostasis, embryonic differentiation and wound repair. Fibronectin is produced by a wide variety of epithelial and mesenchymal cells *in vitro* including: fibroblasts, chondrocytes, myoblasts, Schwann cells, macrophages, hepatocytes and intestinal epithelial cells. Cellular fibronectin is present in many tissues including spleen, lymph node, tonsil, blood vessel walls, liver, kidney, muscle, skin, brain and peripheral nerves. It is found in basement membranes and in loose connective tissue stroma. It is also present in platelet  $\alpha$ -granules and is expressed on the platelet surface after activation.

### Uses

1. Immunohistochemical localization of fibronectin in normal, inflamed and neoplastic tissues.
2. Detection of fibronectin on cultured cells.
3. Structure and function studies of fibronectins in human and animal body fluids, tissues and cells.

Affinity isolated antibody to human fibronectin can be used for immunofluorescent and immunoperoxidase staining of cultured cells, frozen sections and formalin-fixed, paraffin-embedded tissues. Other fixatives, e.g. methacarn and ethanol, may also be used.

**Protein Concentration:** 0.5 mg/ml.

### Working Dilutions

1. A working dilution of 1:1,000 was determined by indirect immunoblotting using human plasma and fibronectin and the Rabbit ExtrAvidin® Staining Kit (Sigma Stock No. EXTRA-3).
2. A working dilution of 1:10,000 was determined by indirect ELISA using 5 µg/ml human fibronectin for coating of microtiter plates, Horseradish Peroxidase Conjugated Goat Anti-Rabbit IgG (Sigma Product No. A-0545) as second antibody and OPD as substrate.
3. A working dilution of 1:400 was determined by indirect immunofluorescence using human foreskin cultured fibroblasts and FITC Conjugated Goat Anti-Rabbit IgG (Sigma Product No. F-9887) as second antibody.

### Storage

For continuous use, store at 2-8°C. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

\* Due to the sodium azide content a material data safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.