

## Certificate of Analysis

### HUMAN FIBRONECTIN

Fibronectin is a broad range natural cell adhesion factor. It is a 440-500 kDa dimeric glycoprotein consisting of two similar 220-250 kDa subunits linked by two disulfide bonds. It is found as a dimer in plasma and in multimeric form in the extracellular matrix and on cell surfaces. Its primary function is related to cell adhesion to the extracellular matrix which occurs via the Arg-Gly-Asp-Ser (RGDS) sequence of fibronectin with the appropriate transmembrane integrin receptor of the cells.<sup>1</sup> Other domains of fibronectin are also involved with this adhesion process and may involve interaction with collagen, heparin and other cell surface glycosaminoglycans.<sup>1</sup> The conformation and orientation of adsorbed fibronectin is also important and has an effect on cell spreading and strength of adhesion of endothelial cells.<sup>2</sup> Fibronectin addition to serum free medium promotes cell adhesion.<sup>3</sup> More significant effects are observed with BHK, CHO and other cell lines by coating of cultureware with fibronectin at 1-5 ug/cm<sup>2</sup>. Details of fibronectin structure, properties, distribution, cellular expression, interaction with other proteins, matrix properties, cell interactions and adhesion and effects on differentiation can be found in two excellent books by Hynes or Moshier.<sup>4,5</sup>

PRODUCT: FIBRONECTIN, Human  
 CATALOG NUMBER: 356008 LOT NUMBER: \_\_\_\_\_  
 SOURCE: Human plasma

**NOTE: The source plasma was tested and found nonreactive for hepatitis B surface antigen (HB<sub>s</sub>Ag) and negative for antibodies to human immunodeficiency virus (HIV), hepatitis C virus (anti-HCV), and syphilis (RPR). Nevertheless, this product should be handled using the same safety precautions used when handling potentially infectious material.**

QUANTITY &  
 PHYSICAL FORM: 5 milligrams per vial, lyophilized.

FORMULATION: 100 mM CAPS, 0.15M NaCl, 1 mM calcium chloride, pH 11.0

RECONSTITUTION  
 & USE:

**TO ENSURE PORPER SOLUBILITY OF HFN THIS RECONSTITUTION PROCEDURE MUST BE FOLLOWED.**

Equilibrate vial to room temperature. Resuspend in five milliliters of sterile distilled water. Allow 30 minutes for material to go into solution. **DO NOT AGITATE OR SWIRL.** If entire amount of material is not to be used immediately, transfer into appropriate aliquots and store at -20°C. It is recommended that solubilized product is used within two weeks. **DO NOT STORE IN FROST-FREE FREEZER. AVOID MULTIPLE FREEZE THAWS.**

Human Fibronectin is generally used in the concentration range of 1-5 micrograms per cm<sup>2</sup> of growth surface for attachment or at 5 micrograms per ml as a media additive.

MOLECULAR WEIGHT: Please see reverse for coating directions.  
 440,000 in non-reduced form.

QUALITY CONTROL:  $\geq$  90% by 4-12% SDS-PAGE under reducing conditions.

Human Fibronectin has been tested for its ability to promote attachment and spreading using BHK-21 cells.

Fibronectin has been tested and found negative for the presence of bacteria, fungi and mycoplasma.

STORAGE: Stable when stored at 2-8°C. **DO NOT FREEZE.**

EXPIRATION DATE:

- REFERENCES:
1. Aota, S., et.al., J. Biol. Chem., **266**:15938 (1991).
  2. Iuliano, D.J., et.al., J. Biomed. Mater. Res., **27**:1103 (1993).
  3. Barnes, D., and Sato, G., Cell, **22**:649 (1980).
  4. Hynes, R.O., Fibronectins, Springer-Verlag, New York (1990).
  5. Mosher, D.F. (ed), Fibronectin, Academic Press, New York (1989).

### Coating Procedure

Use these recommendations as guidelines to determine the optimal coating conditions for your culture system.

- 1) Dilute fibronectin to desired concentration using serum-free culture  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  free medium or buffer at pH 7-9. The final solution should be sufficiently dilute so that the volume added will cover the surface evenly.

Example: If the final coating concentration will be 5  $\mu\text{g}/\text{cm}^2$ , dilute the material to 50  $\mu\text{g}/\text{ml}$  and add 1 ml/35 mm dish, 3 ml/60 mm dish, etc.

**NOTE:** Because of the CAPS component in the HFN preparation, buffers of media containing  $\text{Ca}^{++}$  and/or  $\text{Mg}^{++}$  added to the HFN may result in the formation of insoluble metal hydroxides. This will not occur if the buffering capacity of the diluent brings the pH to 8.0 or lower.

- 2) Add appropriate amount of diluted fibronectin to culture surface.
- 3) Incubate at room temperature for 1 hour.
- 4) Aspirate remaining material.
- 5) Rinse plates carefully with  $\text{dH}_2\text{O}$  - avoid scraping bottom surface.
- 6) Plates are ready for use. They may also be stored at 2-8°C damp or air dried if sterility is maintained.