BI
Biological Industries
Culture of Excellence

LIFE TECH
Life Technologies™
Your Molecular & Cell Technology Partner

Attachment Factors

Attachment factors are structural proteins or protein-like substances that have adherent capabilities and increase cell-substrate interactions in a culture dependent attachment milieu. A number of glycoproteins have been identified that promote and/or influence in vitro cell attachment to the surface or substratum of the culture vessel.

Normal attachment, growth and development of many cell types are dependent on attachment factors and extracellular matrix components. While some cells are able to synthesize these components, others require an exogenous source, particularly when grown in serum-free culture.

The growth and differentiation of anchorage-dependent cells are often strongly influenced by either glass or plastic culture flasks utilized as a substrate. In order to facilitate attachment, cell spreading, growth, morphology, differentiation, and motility of your cells, Biological Industries offers an extensive line of attachment and matrix factors. Each lot is cell culture tested to assess its ability to promote cell attachment and spreading.

Collagen is a major structural protein of extracellular matrix and is the principal protein found in connective tissues. It is found not only in the organic portion of bones, skin, teeth and tendons, but also occurs in other parts of the body as fibrous inclusions. Like other fibrous proteins, collagen is not readily available unless it undergoes heat treatment such as boiling which converts collagen into gelatin. It is an unusual protein, rich in amino acids such as glycine, lysine, proline and others but unfortunately not enough of the essential amino acids. Usually the gelatin derived from collagen is a relatively poor-quality protein.

Gelatin solution (0.1%) is intended for coating cell culture flasks or plates utilized in the growth of Mouse ES cells without a feeder layer. Leukemia Inhibitory Factor (LIF), a pleiotropic, polyfunctional glycoprotein (IL-6) cytokine, should be added to the medium. This impacts growth promotion and prevents cell differentiation on a wide array of various tissue types and target cells.

Human Fibronectin Solution, 1mg/ml

Product Name	Catalogue No.	Unit Size	Storage Temp.
Human Fibronectin Solution, 1mg/ml	05-750-1H	1ml	2-8ºC
	05-750-1F	5ml	2-8ºC

Human Fibronectin (hFN) was tested and found suitable matrix for many cell types as well as for stem cells (e.g. mesenchymal stem cells). Biological Industries' hFN is obtained by affinity purification on gelatine-sepharose from human plasma.

Features
- A complete, ready-to-use solution.
- Suitable for various animal cells.
- Performance tested.

Source
Human plasma.

Concentration
1mg/ml, based on E (1%, 280nm)=12.8.

Identification
A major single band of approx. 220,000 Dalton is evident.

Quality Control
This product has been tested for its ability to promote the attachment and spreading of BHK-21 cells in Serum-free medium.

Suggested Coating Procedures
The recommended concentration of the Fibronectin is 5 mg per ml of medium (or 2-10 µg/cm2). The Fibronectin should be added to DPBS or growth medium in the growth vessel for at least 30 minutes in incubator (37°C). Before seeding, wash the vessel with DPBS or medium. When the medium is replaced in the days following initial seeding no further Fibronectin is required.

References

1. Ruoslahti E. and Ruoslahti. E. Int. J. cancer 20, 1-5 (1977).
2. Miekka S.I e,. al. Thrombosis Research 27, 1-14 (1982).
3. Mosesson MW. and Umfleet RA. The J. of Biological Chemistry Vol.245, No.21 5728-5736 (1970)
4. Vuento M. and Vaheri A. Biochem. J. (1979) 183, 331-337.

Bovine Fibronectin Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
Bovine Fibronectin Solution 1mg/ml	03-090-1-01	1ml	2-8ºC
	03-090-1-05	5ml	2-8ºC

Fibronectin is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells. Fibronectin is particularly useful for the culture of cells that are not capable of synthesizing their own biomatrix or when culturing cells in serum-free medium.

Source
Irradiated citrated bovine plasma.

Description
A clear sterile solution containing Fibronectin, obtained by affinity purification on gelatin-sepharose from bovine plasma. The Fibronectin solution contains buffer salts.

Concentration
1mg/ml, based on E (1%, 280nm) =12.8

Identification
A major single band of approx. 220,000 Dalton is evident.

Suggested coating procedure
The Fibronectin should be added to the growth medium in the culture vessel which is then placed in an incubator 30-60 minutes before seeding. The recommended concentration of the Fibronectin is 5 micrograms per ml of medium. When the medium is replaced in the days following initial seeding, no further Fibronectin is required.

References

1. Ruoslahti E. Int. J. Cancer 20, 1-15 (1977).
2. Miekka S.I Et Al Thrombosis Research 27, 1-14 (1987).
3. Mosesson M.W The J. of Biological Chemistry Vol.245, No.21 5728-5736 (1970).

MSC Attachment Solution

Product Name	Catalogue No.	Unit Size	Storage Temp.
MSC Attachment Solution	05-752-1S	0.1ml	2-8ºC
	05-752-1F	1ml	2-8ºC
	05-752-1H	5ml	2-8ºC

A xeno-free (XF) solution for facilitating attachment and spreading of hMSC in serum-free culture system.

Features
- Ready-to-use solution.
- Without xenogenic components (XF).
- Suitable for hMSCs from various sources.
- Designed for use in serum-free culture systems.

For more information see page 10-11

Collagen Type I, Rat Tail

Product Name	Catalogue No.	Unit Size	Storage Temp.
Collagen Type I, Rat Tail	01-990-100	100mg	2-8ºC

Collagens are a family of highly characteristic fibrous protein found in all multicellular animals and are critical in cell adhesion. Collagen Type I is found in several tissues including skin, connective tissue cartilage and bone.
Collagen Type I is an attachment factor that facilitates the attachment and cytoplasmic spreading of all types of anchorage-dependent cells, when used as a thin layer on a tissue culture surface. As a gel, Collagen I enhances expression of cell-specific morphology and function.

Gelatin Solution 0.1%

Product Name	Catalogue No.	Unit Size	Storage Temp.
Gelatin Solution (0.1%)	01-944-1A	500ml	2-8ºC
	01-944-1B	100ml	2-8ºC

Qualified for Mouse Embryonic Stem (ES) Cells.

Mouse Embryonic Stem Cells (mESCs) are used to generate mouse mutants by gene targeting and blastocyst-mediated transgenesis. Undifferentiated ES cells may be maintained in vitro for extended periods without loss of differentiation capacity when re-implanted back into a blastocyst. Well-established general culture conditions usually require the undifferentiated ES cells to be grown on inactive feeder cell layers or on gelatin-coated plates with Leukemia Inhibitory Factor (LIF) in the culture medium to influence cell growth and function. Growth and differentiation of anchorage-dependent cells are strongly influenced by glass or plastic cultureware offered as a cell-substrate interactive platform. Cell growth rates may be exponentially improved by specialized surface treatments or coating with attachment factors such as Gelatin Solution with LIF.

Application
Used for the attachment of a variety of cell types.

Properties
Sterile, Endotoxin Tested and Cell Culture Tested.
Product is ready to use for plating.

NutriMatrix™ - ECM Coated Plastic Ware

Coated with extracellular matrix (ECM) simulates in vivo conditions.

One of the drawbacks in growing cells In Vitro using conventional tissue culture techniques is that the cells rest on plastic rather than on their natural biological support. This natural support is a complex network of macromolecules known as the extracellular matrix or ECM. ECM holds cells and tissues together and provides a highly organized lattice within which cells can migrate and interact with each other. The matrix plays an active and complex role in regulating the behavior of cells that are in contact with it, influencing their shape, migration, proliferation and metabolic functions. In contrast, cells grown on plastic lose many of their natural differentiated properties due to the lack of interaction with ECM.

ECM is composed of different types of collagen glycosaminoglycans, proteoglycans and glycoproteines[1]. It resembles the vascular subendothelial basal lamina in its organization and macromolecular constituents (fibronectin, lamin, collagen types III, IV and V, and sulfated proteoglycans)[2].

Advantages
Rapid attachment; high plating and cloning efficiencies; rapid proliferation, high saturation density; lower requirements for serum and added growth factors; better response to physiologically occurring hormones; expression of differentiated functions; longer life span for cells; flattening and morphological changes; and improved plating consistency.

Among the cells types showing a favorable response to ECM are human, bovine and other origin.

Research Applications
- **Epithelial Cells:**
  NutriMatrix™ ECM-coated plastic vessels with serum-free medium enable a higher rate of success in growing normal and malignant human epithelial cells from biopsy specimens. ECM induces changes in cell shape not observed in cells grown on plastic or isolated components of the ECM. Cells which for different reasons do not flatten or spread on plastic do so rapidly on ECM.
- **Hormone Secretion Research:**
  NutriMatrix™ ECM-coated plastic vessels with serum-free media support the maintenance and normal function of hormone secreting cells such as pancreatic islet cells, hepatocytes pituitary cells, granulosa cells, etc.
- **Secretion of Cellular Products:**
  The ECM/serum-free medium combination promotes research possibilities on various cellular products.

Hormone Response Research:
ECM effects cell shape and hormone responsiveness. As expected the cells do not respond when maintained on artificial substrata or isolated components of the ECM.

Biotechnology Applications[3]

- Yield and Differentiation:
  The maintenance and growth of differentiated cells on ECM is expected to promote a high yield of various hormones and growth factors in tissue culture.
- Purification:
  Growth of cells in serum-free media will facilitate the purification of various cellular products that are secreted into the medium. Purification will be relatively simple due to the absence of serum proteins.
- Production:
  Large-scale growth of cells on ECM can be performed in bulk cell culture NutriMatrix™ vessels coated with ECM, or on NutriMatrix™ ECM-coated microcarriers. Using these techniques, continuous rather than batch processes can be developed.
- Growth Factor Secretion:
  Growth factors may be produced in better yields by human cells cultured on ECM rather than on plastic and can then be purified and used for research and clinical applications.
- In Vitro Toxicological Testing And Drug Screening:
  The growth of cells on ECM in serum free medium may reduce the cost and simplify the procedure of studying the effect on cells of single drugs, drug combinations and hormones or where a single component is being tested at a time.
- Neurobiology:
  ECM has been shown to support the attachment and maintenance of neurons from various sources and to promote the outgrowth and directed elongation of neurites.

Item	Packaging (unit/Pack)	Catalogue No.
Tissue Culture Dishes 35 mm	5	E-TCP-35
Tissue Culture Dishes 60 mm	5	E-TCP-60
Tissue Culture Dishes 90 mm	5	E-TCP-90
Tissue Culture Flasks 25 cm_2	5	E-TCF-25
Tissue Culture Flasks 80 cm_2	5	E-TCF-80
Microtiter 96-Well Plate	1	E-TCMT-F
4-Well Culture Plate	1	E-TCMW-4
6-Well Culture Plate	1	E-TCMW-6
12-Well Culture Plate	1	E-TCMW-12
24-Well Culture Plate	1	E-TCMW-24
Coverslips (Round, 22 mm)	5	E-TCCS-P22
Four 13mm Coverslips In 4-Well Plate	1	E-TC-IF-13
Eight 12mm Filters in 24- Well Plate	1	E-TC-M-12
Eight Well Lab-Tek Chamber Slide	1	E-LT-8

Storage
NutriMatrix™ ECM-coated plastic vessels are shipped at ambient temperature and should be stored at 2-8ºC upon arrival.

[1] Cancer research 46: 3653 (1986).
[2] Blood 65: 1477 (1985).
[3] Metal Ions in Biology and Medicine. Volume 4 by Philippe Collery. Published by John Libbey Eurotext, 1996.

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com