



**LIFE TECHNOLOGIES (INDIA) PVT. LTD.**

*your molecular & cell technology partner*

*Bringing zest to your research*



**BIOLOGICAL INDUSTRIES**

## Extracellular Matrix Coated (ECM) Coated Plastic Ware

Cultured endothelial cells secrete large amounts of extracellular matrix (ECM) onto the plastic surface during their In Vitro proliferation. This ECM is similar in its chemical composition and organization to naturally occurring basement membranes upon which cells migrate, proliferate and differentiate In Vivo. Thus, disposable plastic ware coated with ECM mimics the In Vivo environment under In Vitro experimental conditions.

Studies carried out over the past decade have indicated that anchorage-dependent cells growing on ECM have a higher proliferation rate, undergo more efficient plating, reach a higher saturation density, exhibit lower requirements for serum and growth factors, and express differentiated functions. In addition, it has also been shown that ECM can promote organ functions In Vitro.

All ECM-coated tissue culture ware products are provided in the form of sterile dry plastic ware ready for use.

The manufacturing process is controlled by a strict quality control system to provide complete standardization of products.

Storage: 2-8°C

Catalogue No.	Product
E-TCP-35	Tissue Culture Dishes 35mm
E-TCP-60	Tissue Culture Dishes 60mm
E-TCP-90	Tissue Culture Dishes 90mm
E-TCF-25	Tissue Culture Flasks 25cm
E-TCF-80	Tissue Culture Flasks 80cm
E-TCMT-F	Microtiter 96-Well Plate (flat)
E-TCMW-4	4-Well Plate
E-TCMW-6	6-Well Plate
E-TCMW-12	12-Well Plate
E-TCMW-24	24-Well Plate
E-TCCS-P22	Coverslips (round) 22mm
E-TC-M-12	Eight 12mm Filters in 24-Well Plate
E-TC-M-30	Four 12mm Filters in 6-Well Plate
E-TC-IF-13	Four 13mm Coverslips in 4-Well Plate

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## **ECM - The Natural Substrate**

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 (1- 6). In contrast, cells grown on plastic lose many of their natural differentiated properties due to the lack of interaction with ECM.

## **ECM Technology**

Growing cells outside the body requires creating conditions that mimic as closely as possible the situation inside the body. Thus, it is essential that anchorage- dependent cells be grown in contact with a natural extracellular framework. Towards this end a new technique for coating tissue culture dishes with a naturally produced basement membrane- like extracellular matrix (ECM) has been developed (7- 11).

During the search for an appropriate tissue culture substratum, it was found that cultured endothelial cells, either vascular or corneal, secrete a large amount of extracellular matrix on to the plastic surface. This material is similar in organization and chemical composition to naturally occurring basement membranes upon which cells migrate, proliferate, and differentiate in vivo. When the endothelial cells are removed they leave the underlying ECM intact and firmly attached to the plastic.

Cells placed in contact with ECM attach rapidly, exhibit high plating and cloning efficiencies, proliferate rapidly, reach a high saturation density, exhibit lower requirements for serum and added growth factors, respond better to physiologically occurring hormones, express differentiated functions, have longer life span, undergo flattening and morphological changes, and have better plating consistency.

In order to improve cell attachment and growth, isolated matrix components such as collagen, laminin and fibronectin have been used to coat tissue culture plastic. However, it has been found that cells placed in contact with ECM adopt growth characteristics, morphological appearance and biological responses which are not expressed when the same cells are maintained on artificial substrata (plastic, glass), even if coated with isolated constituents of ECM such as purified collagen or glycoproteins (7,8,10- 15). The use of naturally produced ECM, assures that the various matrix components will be found in their natural configuration and proportion.

Among the cells types showing a favorable response to ECM are:

### ***Human origin:***

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- Cell lines of Ewing's sarcoma, hepatocarcinoma, choroidal melanoma, cervical carcinoma, breast carcinoma and rhabdomyosarcoma (7,8,20)
- Pituitary tumor (28)
- Epithelial cells derived from surgical biopsies (normal and carcinoma) and malignant effusions breast, ovary, liver, bladder, colon (22,26,27)
- Amniotic fluid cells (23,35)
- Decidual cells (36)

### **Bovine origin:**

- Ovarian granulosa (10,32,34)
- Adrenal cortex (10)
- Vascular endothelium and vascular smooth muscle (11)
- Corneal endothelium
- Lens epithelium

### **Other:**

- Mouse pheochromocytoma; chick and mouse sympathetic and primary sensory cells (12,13)
- Rat oligodendrocytes (14,15)
- Rat pituitary cells (24)
- Rat pancreatic islet beta cells
- Guinea pig keratinocytes

### **ECM Dishes are used like Regular Dishes**

ECM- coated dishes are shipped by express air mail and should be stored at 4degrees C upon arrival. Expiration date one year from completion of production. ECM- coated dishes are handled and used like regular tissue culture dishes.

### **Applications of Extracellular Matrix to Culturing of Epithelial Cells**

Epithelial cells are the principal producers of most hormones, enzymes and other secretory proteins. Epithelial cells also constitute the most common target cells of human cancer. Growth of epithelial cells is therefore of prime importance for various research, clinical and industrial purposes. The main difficulty in obtaining pure and actively growing epithelial cell cultures is that the epithelial cells tend to be overgrown by stromal fibroblasts. Hence, the main requirements for growing primary and early passage epithelial cells are growth conditions that support epithelial out-growth and suppress proliferation of adjoining fibroblasts (16).

Since the removal and dissociation of cells involves surgery and cannot be repeated at will, it is essential to increase the chance of a successful cell plating and growth. Improved plating efficiency and growth rate of such cells are of prime importance when dealing with primary and early passage cell cultures. The growth of primary human epithelial cells derived from surgical biopsies has been previously attempted using media

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supplemented with serum or with conditioned media containing serum (17). Although these media supported epithelial growth, they did not prevent the proliferation of fibroblasts and/or fibroblast- like cells associated with the initial epithelial organoids.

Selective removal of fibroblasts by mesh filtration, centrifugation over Percoll (16), mild trypsinization of the cell layer (23), or growth in medium containing D- Valine (19), minimized the growth of fibroblasts at early stages but failed to prevent the overgrowth of remaining fibroblasts at later stages.

ECM increases plating and cloning efficiency, induces differentiation and enables the growth of epithelial cells in serum- free media. Cells grow on ECM with little or no requirement for serum and added growth factors (20,22,23), thus allowing the use of serum- free media. The use of tissue culture dishes coated with ECM resolves two of the main difficulties in growing epithelial cells.

I. In the case of primary and early passage human epithelial cells, ECM supports high plating efficiency and rate of cell attachment and out-growth (7,8,22).

II. Many epithelial cells which fail to express differentiated properties when grown on plastic retain their normal functioning when plated on ECM.

Examples are nerve cells from which neuronal processes start protruding, and hormone producing pituitary, pancreatic and granulosa cells for growth hormone, TSH, insulin, steroid hormones (1,24,25).

Growth of fibroblasts is prevented by the omission of serum from the culture medium while ECM plated epithelial cells are less affected and continue to proliferate. In the presence of a serum free medium, contaminating fibroblasts did not exceed 3- 5% of the total cell population and were in most cases eliminated by passing the cells from ECM to regular tissue cultures dishes (22).

ECM- coated dishes with serum- free medium enable a higher rate of success in growing normal and malignant human epithelial cells from biopsy specimens. The higher rate of success is due to better plating efficiency and active epithelial cell proliferation in a serum- free medium which suppresses the growth of fibroblasts (22,26- 28).

Whereas serum- free media have been shown to sustain the growth of some human epithelial cell lines, the success rate in using these media for growth of human epithelial cells in primary cultures is very low. This holds true also for epithelial cells from various experimental animals (16,29). ECM enhances the growth of cells in the serum- free environment by reducing there serum requirement and increasing plating and cloning efficiency.

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. These cells are more receptive to hormones and naturally occurring factors.

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## **ECM/Serum Free for Hormones, Enzymes, Growth Factors and Other Cellular Products**

### ***Hormone Secretion Research***

ECM- coated dishes 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. (24,25,32,33). The ability to culture cells from endocrine organs makes it possible to study control mechanisms of hormone production. This is essential for both basic studies on the control of mechanisms of hormone production and secretion and for industrial applications requiring inducement of maximal hormone secretion. Demands for cellular products such as hormones, growth factors and enzymes are increasing for both basic and industrial applications.

### ***Secretion of Cellular Products***

The ECM/serum- free medium combination promotes research possibilities on various cellular products due to the following multiple effects:

- **Inducing differentiation:** Various cells do not maintain their differentiated functions outside the body and therefore do not produce and/or secrete active materials which are produced in vivo. Since secretory cells are mainly epithelial, contact with a basement membrane during growth is required for expression of differentiated functions. The development of appropriate conditions for the maintenance of differentiated cells in culture is a key to study specific functions of hormone secreting cells and for increasing the yield of secreted products.
- **Suppressing fibroblasts:** Serum- free media have been shown to suppress the growth of fibroblasts and hence allow the maintenance of almost pure epithelial cell cultures (21).
- **Purification:** In many cases it is difficult to separate the secreted material from the various proteins present in serum. Since ECM allows the growth of cells in serum-free media, it facilitates the purification of various cellular products from medium lacking most macromolecular contaminants.
- **Increasing yields:** In many instances the minute quantities of materials secreted by cultured cells is a major drawback in the production of cellular materials. Use of serum free medium presents exciting possibilities for increasing the yield of various cellular products through batch processing methods.

### ***Hormone Response Research***

ECM effects cell shape and hormone responsiveness. It is thought that the basal lamina or ECM exerts its permissive effects on cell proliferation and differentiation by modifying cell shape to make it more responsive to hormones and other naturally occurring factors. As expected the cells do not respond when maintained on artificial substrata or isolated components of the ECM (8,9). Growing hormone- responsive cells in serum- free environment will eliminate the effects of other hormones and growth factors which are present in serum, and will allow the study of the isolated effects of a specific growth factor or hormone.

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## Hormone Secreting Cells

Pituitary cells: the pituitary gland secretes a variety of hormones controlling several important functions, ranging from growth and differentiation to reproduction. Of major interest is the production of growth hormone and thyroid stimulating hormone (TSH). Synthesis and secretion of TSH are regulated by the levels of thyroid hormones in the circulation and by the secretion of thyrotropin releasing hormone (TRH) by the hypothalamus. Plating adult rat pituitary cells on ECM enabled the development of pituitary epithelial cell monolayers that retain, for about two weeks in culture, their in vivo hormone secreting and responding characteristics (16). With the aid of ECM- coated dishes it is possible to further clarify the molecular and control mechanisms of TSH and growth hormone production by the pituitary gland.

Pancreatic beta- islet cells: when plated on ECM, beta- islet rat cells respond to glucose and secrete insulin to a higher extent than when coated on plastic.

**Granulosa cells:** ECM enables the growth of rat, bovine and human granulosa cells, which otherwise do not grow well and lose their specific properties. ECM has been shown to promote cell communication and induce higher secretion of steroid hormones by granulosa cells (17,22).

**Hepatocytes:** ECM has been shown to support the attachment and monolayer formation by normal hepatocytes. This will enable studies on the production of specific liver enzymes, hormones (transferin) and proteins (33).

## Biotechnical Applications

- 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 (9).
- 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 vessels coated with ECM, or on ECM- coated microcarriers. Using these techniques, continuous rather than batch processes can be developed.
- Variety of products: among the most appealing products are growth hormone and plasminogen activator.
- 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.

## Other Applications

In vitro toxicological testing and drug screening - alternative to laboratory animals: the ability to grow primary cells in vitro for drug screening and toxicological testing may offer an alternative to the use of laboratory animals. 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.

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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. It has also been shown to induce the proliferation and network formation of adult rat brain oligodendrocytes maintained in a serum-free medium (12- 15).

### **ECM PLASTIC WARE IS USED LIKE REGULAR CULTURE VESSELS**

ECM-coated plastic vessels are shipped by express air mail and should be stored at 4°C upon arrival. Expiration date is one year from completion of production. ECM-coated dishes, flasks and slides are handled and used exactly like regular tissue culture plastic ware.

### **REFERENCES**

1. Reid, LM and Rojkind, M (1979) *Methods in Enzymol.* 58, 263- 278.
2. Grobstein, C (1967) *Natl. Cancer Inst. Monograph*, 26, 279- 299.
3. Wessels, NK (1964) *Proc. Natl. Acad. Sci., USA*, 52, 252- 259.
4. Kratochwil, K (1972) In: *General properties in tissue interaction in carcinogenesis*, Ed. Tarin, D., Academic Press, New York, 1- 48.
5. Folkman, J and Moscona, A (1978) *Nature*, 273, 345- 349.
6. Hay, ED (1981) *J. Cell Biol.*, 91, 205- 223.
7. Vlodavsky, I, Lui, Gâ M, and Gospodarowicz, D (1980) *Cell*, 19, 607- 616.
8. Gospodarowicz, D and Vlodavsky, I (1982) *Pro. Cancer Res. There.* 22, 73- 104.
9. Gospodarowicz, D and Ill, CR (1980) *Proc. Natl. Acad. Sci.,USA*, 77, 2726- 2730.
10. Gospodarowicz, D, Delgado, D, Vlodavsky, I (1980) *Proc. Natl. Acad. Sci., USA*, 77, 4094- 4098.
11. Gospodarowicz, D and Ill C.R. (1980) *J. Clin. Inv.*, 65, 1351- 1364.
12. Vlodavsky, I, et al. (1982) *Develop. Biol.*, 93, 285- 300.
13. Lander, AD, Fuji, DK, Gospodarowicz, D and Reichardt, LF (1982) *J. Cell Biol.*, 94, 574- 585.
14. Lubetzkiâ Korn, O, et al. (1983) *Brain Res.*, 267, 151- 155.
15. Ovadia, H, et al. (1984) *Brain Res.*, 322â 93- 100.
16. Owens, RB, Smith, HS, Nelson- Ress, WA and Springen, EL (1976) *J. Nat. Cancer Inst.*, 56, 843- 849.
17. Stampfer, MR, Hallows, RC and Hackett, AJ. (1980) *In vitro*, 26, 415- 425.
18. Yang, J, et al. (1980) *Proc. Nat. Acad. Sci. USA*, 77, 2088- 2092.
19. Gilbert, SE and Migeon, BR (1975) *Cell*, 5, 11- 17.
20. Gospodarowicz, D, Lui, Gâ M, and Gonzales, R (1982) *Cancer Res.*, 42, 3704- 3713.
21. Barnes, D and Sato, GH (1980) *Anal. Biochem.* 102, 255- 270.
22. Biran, S, Horowitz, AT, Fuks, Z and Vlodavsky, I (1983) *Int. J. Cancer*, 31, 552- 566.
23. Vlodavsky, I, Voss Yarkoni, A and Fuks, Z (1982) *Prenatal Diag.*, 2, 13- 23.
24. Sira, O, et al. (1983) *Acta Endocrinol.*, 104, 279- 286.
25. Oliver, C and Kleinman, HK (1984) *J. Cell Biol.* 99, 159a.
26. Pode, D, et al. (1985) *J. Urol.*
27. Crickard, K, Crickard, U and Yoonessi, M (1983) *Cancer Res.*, 43, 2762- 2727.

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28. Bethea, CL and Weiner, RI (1981) *Endocrinology*, 108, 357- 360.
29. Rafferty, KA (1975) *Adv. Cancer Res.*, 21, 249- 272.
30. Vlodavsky, I, et al. (1983) *Cancer Res.*, 43, 2704- 2711.
31. Selby, P, Buick, RN and Tannock, LA (1983) *N. Engl. Med.*, 308, 129- 134.
32. Savion, N, Lui, G-M, Laherty, R and Gospodarowicz, D (1981) *Endocrinology*, 109, 409- 421.
33. Enat, R, et al. (1984) *Proc. Natl. Acad. Sci., USA*, 81, 1411- 1415.
34. Amsterdam, A, Azrad, A, Gordon, S and Furman, A (1984) *J. Cell Biol.* 99, 159a.
35. Crickard, K and Golbus, MS (1982) *Prenatal Diag.*, 2, 89- 95.
36. Hochnerâ Celnikler, D, et al. (1984), *Biol. Reproduction* 31, 827- 836.

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