ProductInformation

SigmaScreen™
Fibronectin Coated Multiwell Plates
Format: 96-well, clear/clear bottom

Product Number **S 3815**

Product Description

The presence of one or more components of the extracellular matrix (ECM) on the growth surface can enhance cell attachment and proliferation in certain cell lines.[1] The glycoprotein fibronectin, which is a major component of the ECM, has cell adhesion properties which make it a widely used attachment factor on tissue culture substratum.[3] Cellular adhesion occurs primarily via an interaction of the Arg-Gly-Asp (RGD) sequence of fibronectin with a family of integrin receptors located on the cell surface.[2]

Serum supplementation in cell culture media provides the necessary growth and attachment factors required for many cell lines. However, the undefined and inconsistent properties of serum limit the use of serum-dependent cultures in generating standardized and controlled experimental results. The SigmaScreen Fibronectin Coated Multiwell Plates provide a superior cell environment that promotes cell-plating efficiency, improves morphology, and enhances cell proliferation in low serum or even serum-free conditions compared to tissue culture treated plates.

SigmaScreen Fibronectin Coated Multiwell Plates are prepared with human plasma fibronectin (Product No. F 2006). They are tested for cell attachment and spreading in serum-free Dulbecco's Modified Eagle's Medium using BHK-21 hamster kidney cells and are manufactured in a highly controlled environment. Each lot is tested for the presence of endotoxins, bacteria, and fungi.

Applications

- Cell culture in low serum or serum-free media
- Promotion of attachment and spreading of many normal and transformed cells including epithelial, endothelial, neuronal, and ovarian cells
- Improved survival of primary cultures
- Evaluation of the effects of fibronectin on cell genotype and phenotype

The following is a partial list of cells that have been reported to be successfully cultured on Fibronectin coated surfaces:

Epithelial Cells

- Human breast adenocarcinoma cells (MDA MB 231 and MCF-7)[6,15]
- Human epidermoid carcinoma cells (A-431)[15]
- HeLa cells[13]
- Caco-2 cells[7]
- Human embryonal kidney cells (293)[8,11]
- Human lung adenocarcinoma cells (A549)[8]
- Human fibrosarcoma cells (HT-1080)[8]

Endothelial Cells

- Primary Human Umbilical Vein Endothelial Cells (HUVEC)[5,15]
- Bovine aortic endothelial cells[15]

Hepatocytes

- Primary rat hepatocytes[9]
- Primary human hepatocytes[12]

Other cells:

- BHK-21 cells
- CHO-K1 cells[8,10,15]
- Primary neural crest cells[14]
- Murine neuroblastoma cells (41A3)[4]
- Rat pulmonary artery smooth muscle cells (PAC-1)[15]
- Human lung fibroblast cells (WI-38)[15]
- Human osteosarcoma cells (MG-63)[8]

Precautions and Disclaimer

For research use only, not intended for use in diagnostic procedures.

Storage/Stability

The 5 plate package is in a resealable bag with individual lids and desiccant.

For optimal performance, the unopened product should be stored in a dry place at 2-8 °C. Do not freeze. The product should not be exposed to temperatures above 50 °C.

Refer to the Certificate of Analysis for the expiration date. The Certificate of Analysis can be obtained from the Sigma-Aldrich website (www.sigma-aldrich.com). Once opened, the product should be used promptly.

Procedure

1. Using standard protocols, harvest the cells.
2. Collect the harvested cells by centrifugation at 200 to 300 x *g* for five minutes. Discard the supernatant.
3. Resuspend the cell pellet in fresh, complete medium.
4. Count the cells. Dilute in complete medium for a cell stock suspension of 1 to 50 x 10^5 cells/ml.
5. Open the plate packaging under aseptic conditions. Pipette 100 µL of the cell stock suspension into each well of the 96 well plate. If necessary, add appropriate quantities of test substances to the wells.
6. Cover the plates with lids. Place in a 37 °C, 5% CO_2, humidifying incubator for the desired amount of time.
7. Observe the cell morphology with a microscope.
8. Gently aspirate to remove the medium and non-adherent cells from the wells. Gently wash with 100 µL Dulbecco's PBS (Product No. D 8662) per well. Repeat this wash step, using care not to dislodge the cells.
9. Using standard protocols, lyse the cells. A lysis reagent such as CelLytic™-M Mammalian Cell Lysis/ Extraction Reagent (Product No. C 2978) may be used.
10. Add 175 µL of Bradford Reagent (Product No. B 6916) to each well. Mix thoroughly and incubate at room temperature for 10-20 minutes.
11. Read the absorbance at 595 nm. Calculate the protein content of the attached cells by comparing the sample absorbance with an appropriate standard curve.

Product Profile

Table 1 – Plate Features

Property	96 Well Plate
Plate composition	Tissue culture treated polystyrene
Lid	Yes
Well configuration	Flat bottom/round
Well width	6.4 mm
Well depth	11 mm
Maximum recommended working volume, per well	250 µL
Recommended culture volume, per well	100 µL
Growth area, per well	32 mm^2
Coating coefficient of variability, well-to-well	≤ 10%

References

1. Freshney R. (1987). Culture of Animal Cells: A Manual of Basic Technique, Alan R. Liss., New York.
2. Ruoslahti, E., et al., New perspectives in cell adhesion: RGD and integrins. Science, **238**, 491-497 (1987).
3. Kleinman, H.K., et al., Use of extracellular matrix components for cell culture. Analytical Biochemistry, **166**, 1-13 (1987).
4. Arcangeli, A., et al., Integrin-mediated neurite outgrowth in neuroblastoma cells depends on the activation of potassium channels. J. Cell Biol., **122**, 1131-1143 (1993).
5. Moser, R., et al., Migration of primed human eosinophils across cytokine-activated endothelial cell monolayers. Blood, **79**, 2937-2945 (1992).
6. Ronnov-Jessen, L., et al., Identification, paracrine generation, and possible function of human breast carcinoma myofibroblasts in culture. *In Vitro* Cell. Dev. Biol., **28A**, 273-283 (1992).
7. Kuwada, S., et al., Integrin α5/β1 mediates fibronectin-dependent epithelial cell proliferation through epidermal growth factor receptor activation. Mol. Biol. Cell, **11**, 2485-2496 (2000).
8. Munger, J., et al., Interactions between growth factors and integrins: Latent forms of transforming growth factor-β are ligands for the integrin αvβ1. Mol. Biol. Cell, **9**, 2627-2638 (1998).
9. Sanchez, A., et al., Fibronectin regulates morphology, cell organization, and gene expression of rat fetal hepatocytes in primary culture. J. Hepatol., **32**, 242-250 (2000).
10. Matter, M., et al., A signaling pathway for the $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins that elevates *bcl-2* transcription. J. Biol. Chem., **276**, 27757-27763, (2001).

11. Quattrocchi, C., et al., Reelin is a serine protease of the extracellular matrix. J. Biol. Chem., **277**, 303-309 (2002).
12. Vlaheta, R. et al., Dedifferentiation of human hepatocytes by extracellular matrix proteins in vitro: quantitative and qualitative investigation of cytokeratin 7, 8 , 18, 19 and vimentin filaments. J. Hepatol., **28**, 677-690 (1998).
13. Toshima, J. Cofilin phosphorylation by protein kinase testicular protein kinase 1 and its role in integrin-mediated actin reorganization and focal adhesion formation. Mol. Biol. Cell, **12**, 1131-1145 (2001).
14. Monier-Gavelle, F., et al., Cross talk between adhesion molecules: Control of N-cadherin activity by intracellular signals elicited by β1 and β3 integrins in migrating neural crest cells. J. Cell Biol., **137**, 1663-1681 (1997).
15. Twal, W. et al., Fibulin-1 suppression of fibronectin-regulated cell adhesion and motility. J. Cell Sci., **114**, 4587-4598 (2001).

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