

## **SpongeCol<sup>®</sup>, Collagen Sponge**

COLUMAR PORE ARCHITECTURE STRUCTURE, 21 MM DIAMETER  
Catalog Number 5135-5EA

### **Product Description**

*SpongeCol<sup>®</sup>* is a collagen sponge with an interpenetrating, columnar porous architecture structure. *SpongeCol<sup>®</sup>* contains a unique columnar porous network which permits cells and nutrients to flow completely through the interpenetrating pores and provides an increased surface area for cell attachment, growth and migration. *SpongeCol<sup>®</sup>* is composed of highly purified Type I collagen which best supports the attachment, proliferation, and function of cells. The collagen sponge is lightly cross-linked for increased mechanical strength and durability for short and long term tissue culture yet is still biodegradable over the longer-term. The diameter of the pores ranges from 100 to 400 micron with the average diameter being approximately 200 microns. The collagen disc is approximately 21 mm in diameter and 1.5 mm thick. The sponge discs fits into a 12 well culture plate or sanitary luer connectors for flow perfusion. Each package contains five collagen sponge discs. This product is terminally sterilized and ready-to-use.

### **Characterization**

**Pore Size:** Approximately 200 micron pore diameter with a range of 100 to 400 micron.

**Dimensions:** *SpongeCol<sup>®</sup>* is a disc shape that is approximately 21 mm diameter and 1.5 mm thick.

**pH:** Approximately 7.0 to 7.4 when suspended in PBS or tissue culture media

**Endotoxin:** Collagen used to produce this product had an endotoxin level of  $\leq 1.0$  EU/ml

**Sterility:** *SpongeCol<sup>®</sup>* is irradiated and has passed sterility testing.

**Storage/Stability:** Room Temperature - Heating above 40 °C is not recommended. Store in a cool, dry place. The stability of the product is under evaluation.

### **Precautions and Disclaimer**

This product is for R&D use only and is not intended for human or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### **Preparation and Usage**

#### **A. Preparation and Seeding:**

Note: Cell attachment to the sponge is generally the most critical step in tissue culture. Temperature, pH, gas exchange and cell concentration can affect the rate and efficiency of attachment. Optimum seeding rate depends on the type of cell being cultured.

1. Aseptically remove the sponge discs from the packaging in a laminar flow work station.
2. Carefully place the sponges into the wells of a 12 well tissue culture plate using a sterile instrument. Be careful not to damage the sponge as it is being transferred. It is recommended to use non-treated tissue culture plasticware.

Note: Tissue-coated plasticware may need to be coated with agarose to prevent cell attachment to the plastic instead of the sponge.

3. Suspend cells at desired concentration ( $1 \times 10^4$  –  $1 \times 10^5$  cells/mL) and dispense sufficient volume of cell solution on top of the sponge placed in the well.

Note: An alternative method is to suspend cells in a neutralized collagen solution (such as PureCol<sup>®</sup> type I collagen Catalog #5005-100ML or PureCol<sup>®</sup> EZ Gel Catalog #5074-35ML). Dispense collagen/cell solution on top of the sponge placed in the well.

4. Transfer to a 37°C incubator for about 1 - 2 hours to allow for initial cell attachment.

Note: If using the collagen suspension method, the collagen will polymerize at 37°C and encapsulate your cells within the collagen matrix and the sponge.

5. After 1 - 2 hour, remove the plate from the incubator and check for cell attachment. Additional testing may be required to optimize the time it takes for the cells to attach to the sponge. Check the morphology of the cells. Cell adherence and spreading will dictate the time for attachment.
6. Once the cells have adequately attached to the sponge, increase the final volume in each well to fully cover and provide adequate medium for the culture system.

#### **B. Changing the Media:**

1. Change the media 12 to 24 hours after the initial seeding. The frequency of changes will be determined by cell type, cell attachment efficiency, pH (maintain at pH 7.0 to 7.4) utilization of medium nutrients available to cultures. More frequent medium changes may be required compared to 2D culture systems.

#### **C. Harvesting of Cells:**

Note: Protease digestion is the standard method of releasing cells from the sponges. The strength of the attachment of the cells to the collagen sponges will vary from cell line to cell line. The enzyme concentration and digestion time will vary depending upon the activity of the enzyme and the confluence of the cells. Collagenase and/or trypsin may be the preferred method.

1. Washing the sponge with EDTA-PBS may assist the protease digestion. Add sufficient volume to cover the sponge.
2. Aspirate the EDTA-PBS solution from the well.
3. Add sufficient dissociation solution to the well to fully over the sponge.
4. Transfer to a 37°C incubator. Check for cell detachment periodically for cell detachment.
5. Once the cells have fully detached, remove the cells and dispense in a centrifuge tube.
6. Centrifuge the cells as require.

#### **REFERENCES**

1. Park, H., Radisic, M., Lim, J.O., Chang, B.H., and Vunjak-Novakovic, G., A novel composite scaffold for cardiac tissue engineering. *In Vitro Cellular & Developmental Biology–Animal*, 41:188-196, 2005.
2. Markowicz, M., Koellensperger, E., Neuss, S., Steffens, G.C.M., and Pallua, N., Enhancing the vascularization of three-dimensional scaffolds: new strategies in tissue regeneration and tissue engineering. *Topics in Tissue Engineering*, 2:1-14, 2005.
3. Aoki, S., Toda, S., Ando, T., and Shgihara, H., Bone marrow stromal cells, preadipocytes, and dermal fibroblasts promote epidermal regeneration in their distinctive fashions. *Molecular Biology of the Cell*, 15:4647-4657, 2004.
4. Wendt, D., Marsono, A., Jakob, M., Heberer, M., and Martin, I., Oscillating perfusion of cell suspensions through three-dimensional scaffolds enhances cell seeding efficiency and uniformity. *Biotechnology and Bioengineering*, 84, No. 2:205-214, 2003.
5. Von Heimburg, D., Zachariah, S., Heschel, I., Kuhling, H., Schoof, H., Hafemann, B., and Pallua, N., Human preadipocytes seeded on freeze-dried collagen scaffolds investigated in vitro and in vivo. *Biomaterials*, 5:429-38, 2001.
6. Alini, M., Li, W., Markovic, P., Aebi, M., Spiro, R.C., and Roughley, P.J., The potential and limitations of a cell-seeded collagen/hyaluronan scaffold to engineer an intervertebral disc-like matrix. *Spine*, 5:446-54; discussion 453, 2003.
7. Lu, Q., Ganesan, K., Simionescu, D.T., and Vyavahare, N.R., Novel porous aortic elastin and collagen scaffolds for tissue engineering. *Biomaterials*, 22:5227-5237, 2004.