COSMO BIO CO., LTD.
Inspiration for Life Science

Sample

AteloCell®

Atelocollagen, Honeycomb disc 96

Cat No.:	KOU-CSH-96	
Lot No.:	*	
ORIGIN:	Bovine dermis	
STORAGE:	Room Temperature	
REFERENCES:	Refer to the AteloCell® website http://www.cosmobio.com	
	Specification	Results
DIAMETER OF PORES:	200～400 μm	Pass
STERILITY TEST: (Medium: TGC-I and SCD)	Negative	Pass
CELL CULTURE TEST: (Cell: Human Fibroblast)	Normal	Pass

FOR RESERCH USE ONLY, NOT FOR HUMAN BODY.

Manufactured by KOKEN Co., Ltd.

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Instruction manual for Atelocollagen, Honeycomb sponge

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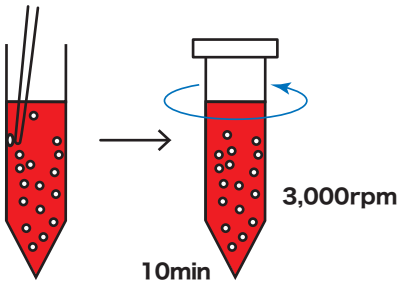
Ⅰ .Defoaming*1 Atelocollagen, Honeycomb sponge

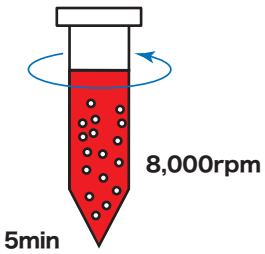
A.Defoaming method 1

(1) Soak Honeycomb in cell culture medium or PBS and gently squeeze the sponge using forceps or a pipette tip.
(2) Centrifuge at 3,000 rpm for 10 min.



B. Defoaming method 2

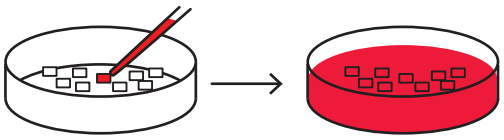
(1) Soak Honeycomb in cell culture medium or PBS.
(2) Centrifuge at 8,000 rpm for 5 min.



*1: Insufficient defoaming will prevent cells from seeding into the pores of Honeycomb.

Ⅱ . Seeding cells to Atelocollagen, Honeycomb sponge

(1) Place defoamed Honeycomb into each well of a 96-well plate.
(2) Slowly apply the cell suspension onto Honeycomb while gently squeezing with a pipette tip*2
(3) Fill each well with cell culture medium.



※2 To increase cell attachment, use collagen-containing medium prepared by mixing medium and an acidic collagen solution (IPC-50 or IAC-50). Refer to the instructions for preparing collagen solutions for details.

Ⅲ . Mass culture with Atelocollagen, Honeycomb sponge

1. Defoaming Hoenycomb

Method1

(1) Add 10 mL of culture medium or PBS to a bottle containing Honeycomb. Most Honeycomb will float on the medium due to entrapped air.
(2) Transfer Honeycomb to a 100 mL spinner flask containing 90 mL of cell culture medium or PBS
(3) Defoam Honeycomb by stirring at 40 rpm at 4℃ for 24 hours.

Method 2

(1) Add Honeycomb to a centrifuge tube containing cell culture medium or PBS.
(2) Defoam Honeycomb by centrifuging at 1,000 rpm for 10 min.

Method 3

(1) Add 15 mL of cell culture medium or PBS to a bottle containing Honeycomb.
(2) Defoam Honeycomb by compressing with a stick.

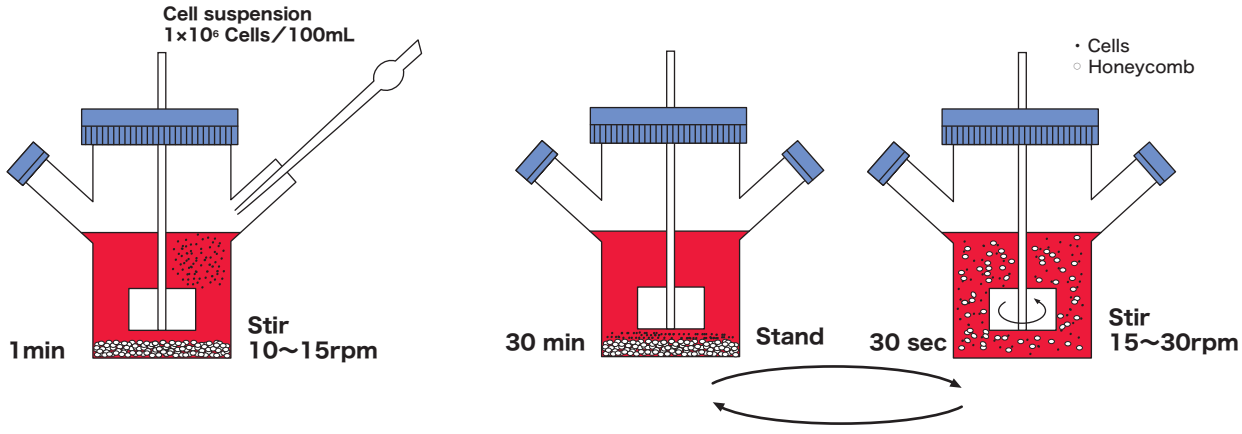
2. Seeding cells

(1) When using method 2 or 3, replace the cell culture medium or PBS with fresh medium or PBS. Use 100-200 mg of Honeycomb for 100 mL of cell culture medium. This weight of Honeycomb will have a volume of about 15-30 mL when swollen.
(2) Seed about $1x10^6$ cells/100 mL of cell culture medium.
(3) After stirring at 10-15 rpm for one minute, allow to stand. Stir for 30 seconds every 30 minutes (15-30 rpm). Repeat this process for two hours to allow the cells to adhere to Honeycomb.

3.Culturing cells

After confirming cell adhesion to Honeycomb, stir the cell culture medium gently to move Honeycomb very slowly in the medium.

Seeding cells



Cell Growth Curve of F-7000 (human fibroblast) cells on Honeycomb.

A typical cell growth curve is shown below. Honeycomb can help provide a cell density of approximately $7x10^6$-10^7/mL in 2 weeks.