COSMO BIO CO., LTD.
Inspiration for Life Science

KOKEN

AteloCell®

Certificate of Analysis

3D Honeycomb Boosted Cat No. 3D-HCB
Lot No. ******

COLLAGEN ORIGIN:	Bovine dermis
COLLAGEN TYPE:	Type I atelocollagen
SIZE:	Φ5 mm x 5 mm
DIAMETER OF PORES	100～300 μm
FORM:	Immersed in PBS
STORAGE:	2～10°C
PRECAUTIONS:	Do not lay down the bottle on its side.

For instruction manuals in Japanese, please visit the AteloCell® website.
https://atelocollagen.com/atelocell/atelocell_category/manual/

English instruction manuals are available through your local distributors.

	Specifications	Results
STERILITY TEST: (Medium: TGC-I and SCD)	Negative	Pass
CYTOTOXICITY TEST:	Negative	Pass

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

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Instructions for using 3D Honeycomb Boosted

Storage conditions

Store at 2~10℃

Preliminary preparations for cell seeding

Required Materials (not included)

For 1-A	Any tweezers	1 pc.
	Sterile gauze	Appropriate amount
For 1-B	Flat tip tweezers	1pc.

*1 : Since 3D Honeycomb Boosted tends to adhere to the tweezers after compressing described in 1-B, 1-A is more suitable when large number of 3D Honeycomb Boosted are handled..

Procedure

(1-A) Using any tweezers, place a cylindrical 3D honeycomb Boosted in the orientation shown in Fig. 1 on sterile gauze to remove PBS as shown in Fig. 2.

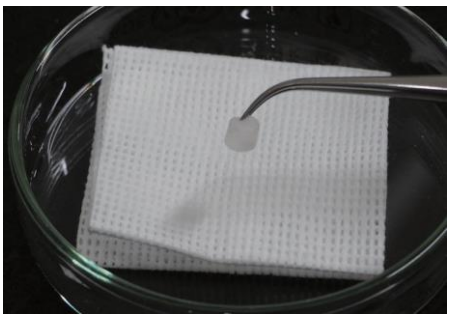

Fig. 1. 3D honeycomb Boosted prior to dehydration

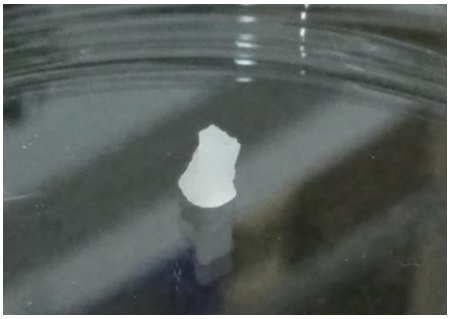

Fig. 2. 3D honeycomb Boosted after dehydration

(1-B) Using flat tip tweezers, grasp the cylindrical 3D honeycomb Boosted in the orientation shown in Fig. 3 and compress it to remove PBS as shown in Fig. 2.

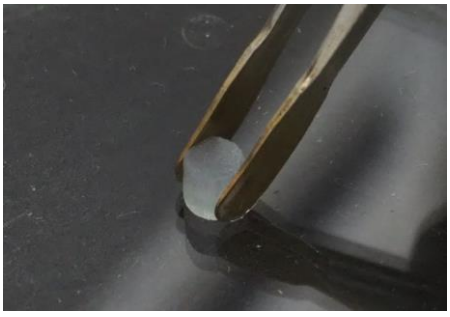

Fig. 3. 3D honeycomb Boosted prior to dehydration

(2) Place the PBS-removed sponge in a cell cultureware such as a 96-well plate in the orientation in which the cylindrical sponge stands free-standing.

Cell seeding Procedure

(1) Slowly drop a 50 µL cell suspension and confirm that it returns to its original cylindrical shape as shown in Fig. 4.

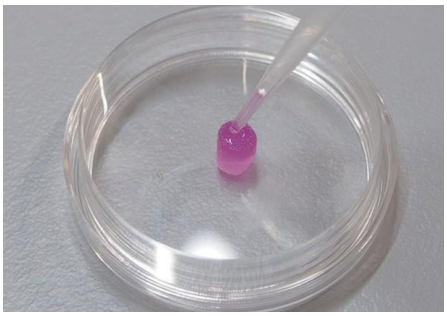

Fig. 4. 3D honeycomb Boosted after cell-seeding

*1: Currently, we have confirmed that cells ranging from 1 × 10^4 to 1×10^6 cells can be cultured.

(2) After incubating the cell seeded sponge for 4 hours to 1 day, add the medium so that 3D Honeycomb Boosted is sufficiently immersed.

e.g.) 96 well plate: Around 150 µL
24 well plate: Around 1 mL
35 mm dish: Around 3mL

*2: If the medium needs to be added immediately after cell seeding, add it slowly and do not hit the 3D Honeycomb Boosted so that the seeded cells do not flow out. .

*3: For example, when 3D Honeycomb Boosted are seeded with 1×10^4 cells and cultured in 96-well plates, it reaches to a limit of cell culture about 1 week later because nutrition in culture medium becomes insufficient, even if you exchange the culture medium several days after incubation. Consider to use larger size cultureware either part way through or from the bigining depending on the number of cells seeded and the culture period.

Instructions for using 3D Honeycomb Boosted

Cell collection

Materials to be supplied by the users

Tweezers	1 pc.
Sterile gauze	Appropriate amount
PBS (-)	Appropriate amount
Trypsin	Appropriate amount

Procedure

*1: The following is a procedure for collecting cells from a single 3D Honeycomb Boosted, using 35 mm dish as an example. Please adjust volume accordingly for other container sizes

(1) Place 4 mL of PBS (-) in a 35 mm dish.
(2) Place 3D Honeycomb Boosted after culture on top of sterile gauze to remove culture medium.
(3) Place 3D Honeycomb Boosted in PBS (-) prepared at (1) .
(4) Place 3D Honeycomb Boosted over sterile gauze to remove PBS (-).
(5) Repeat steps (3) to (4) four times until the redness of the culture medium is removed from 3D Honeycomb Boosted.
(6) Place 3D Honeycomb Boosted in a 1.5 mL tube. Add 100 µL of trypsin directly on 3D Honeycomb Boosted and incubate at 37℃ and 5% CO_2 for 5 minutes.
(7) Add 1 mL of medium to pass through the inside of the pores of 3D Honeycomb Boosted, pass the same medium through the inside of the pores several times, and then collect it in a 15 mL centrifuge tube.
(8) Repeat (7) again.
(9) Centrifuge the 2 mL of cell suspension in a 15 mL centrifuge tube at 1000 rpm for 3 minutes at room temperature and remove the supernatant by gentle aspiration with a pipette.
(10) Suspend the cell pellet in an appropriate volume of medium.

Instructions for using 3D Honeycomb Boosted

Preparation of DNA quantitation (preliminary preparation)

Materials to be supplied by the users

Tweezers	1 pc.
Sterile gauze	Appropriate amount
Triton™ X-100	Appropriate amount
Proteinase K	Appropriate amount

Procedure

(1) Add 400 µL of 0.2% Triton™ X-100 to 1.5 mL tube.

(2) Place 3D Honeycomb Boosted after culture on top of sterile gauze to remove medium.

(3) Place 3D Honeycomb Boosted in the 1.5 mL tube prepared at (1) and vortex briefly.

(4) Attach a cap lock and incubate in a 100℃ water bath for 10 minutes.

(5) Allow to stand until it cools to room temperature, then spin down.

(6) Remove the cap lock, add 30 µL of Proteinase K, and vortex briefly.

(7) Reattach the cap lock and incubate in a 56℃ water bath until 3D Honeycomb Boosted is dissolved.

(8) Subsequently, use DNA-quantification reagents such as PicoGreen® as described in its instructions for use.

RNA collection from 3D Honeycomb Boosted

(1) After culture, place 3D Honeycomb Boosted on top of sterile gauze to remove medium.

(2) Use commercially available RNA extraction kits as described in its instructions for use.

*1: Perform homogenization as appropriate for the product to be used.

Histological evaluation after culture

(1) Since there are no precautions that are unique to collagen, general histological sample preparation is applicable.