KOKEN

Sample

AteloCell®

# Collagen sponge for 35mm culture dish

| | | |
|---|---|---|
| Cat No.: | KOU-CS-35 | |
| Lot No.: | * | |
| ORIGIN: | Bovine tendon | |
| STORAGE: | Room Temperature | |
| REFERENCES: | Refer to the AteloCell® website http://www.cosmobio.com | |
| | Specification | Results |
| STERILITY TEST: (Medium: TGC-I and SCD) | Negative | Pass |
| CELL CULTURE TEST: (Cell: Human Fibroblast) | Normal | Pass |

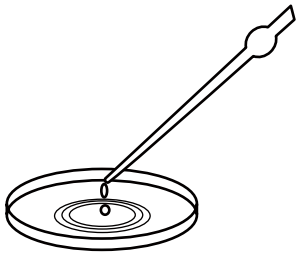
FOR RESERCH USE ONLY, NOT FOR HUMAN BODY.

Manufactured by KOKEN Co., Ltd. KOKEN

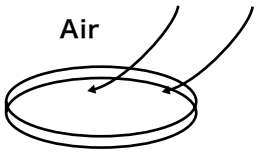
COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME, KOTO-KU, TOKYO 135-0016 JAPAN
TEL: (81)3-5632-9617 / FAX: (81)3-5632-9618 / e-mail: export@cosmobio.co.jp / URL:www.cosmobio.com

## Ⅰ. Culture in collagen-coated dish

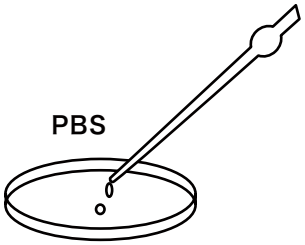
(1) Add a proper quantity (1mL-2mL for 35mm dish Note 1) of AteloCell™ I-AC (Native collagen) or I-PC (Atelocollagen) , either 3mg/mL or 5mg/mL to culture dish and spread over the entire dish.



(2) Open the lid of culture dish in a clean bench and air-dry it at below 25℃.

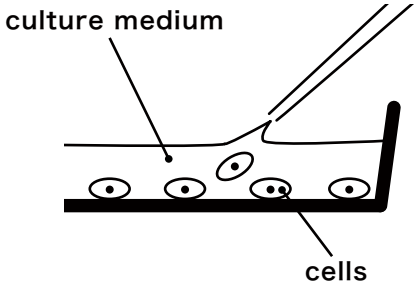



(3) Neutralize the dish by washing several times with PBS (150mM NaCl, 20mM $Na_2HPO_4$, pH7.4) .




(4) Add cell suspension, and culture as usual.

Note 1: Due to the high viscosity of collagen solution, collagen will not spread over the whole culture dish with below 0.5mL for 35mm culture dish and below 1mL for 50mm culture dish. If smaller amounts of collagen is desirable, dilute collagen solution with distilled water adjusted to pH3 with HCl.



## II . Preparation of collagen gel medium

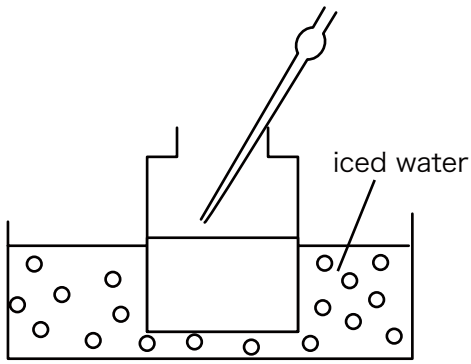
### A. When AteloCell™ I-AC(Native collagen) or I-PC(Atelocollagen) is used.

Reagents

1 Medium (10x concentration )Note 2 ····· 1mL
2 HEPES pH7.4 (100x concentration) ····· 0.1mL
3 $NaHCO_3$ (100x concentration) ····· 0.1mL
4 Distilled water ····· 0.8mL
5 AteloCell™ I-AC( Native collagen) or I-PC (Atelocollagen) ····· 8mL
(3mg/mL or 5mg/mL)

Detailed reagent information

1. Medium (10x concentration: 10 times of concentration than the one normally used at powder medium) Note 2···1 mL
2. HEPES pH7.4 [1 – 5, Prepare the final concentration of mixture at 10mM. So the final concentration of this solution is 10 mM × 100=1000M···0.1 mL
3. $NaHCO_3$ [1 – 5, Prepare the final concentration of mixture at 10mM. So the final concentration of this solution is 10 mMx100=1000mM···0.1 mL
4. Distilled water···0.8 mL
5. AteloCell™ I-AC(Nativecollagen) or I-PC(Atelocollagen)(3mg/mL or 5mg/mL)···8mL




1) Add 1 through 4 above in an ice water bath and then add 5. Collagen solutions are very viscous; when adding, pipette several times to remove the collagen attached to the pipette wall. Be careful not to form air bubbles. Once formed, air bubbles are difficult to remove. The final collagen concentration is about 0.4% when 0.5% solution is used and about 0.24% when 0.3% solution is used. Solidity of collagen gels depend on the final collagen concentration.
2) If necessary, add serum to this mixture at 2 – 4˚C

Note 2: Hanks' medium or Eagle's MEM can easily dissolve in water at 10× concentrations, but it is difficult for rich media such as Dulbecco's modified Eagle's MEM. For such media, prepare 5× solution but maintain the final volume of collagen gel media by changing collagen or distilled water volumes. When preparing gels with a lower collagen concentration, reduce the volume of collagen solution (5) and replace the reduced volume with distilled water. The minimum concentration of collagen required for gel formation is 0.1%.

### B. When AteloCell™ neutral solution(Atelocollagen Eagle's MEM, Hanks' Medium, DMEM are used.

1) Normally above products are kept at frozen conditions, so first thaw the products.
Thaw the products by stirring in 25˚C of warm water bath. It may be gelatinized partially without stirring.

2) Once thawed, put in ice water bath.

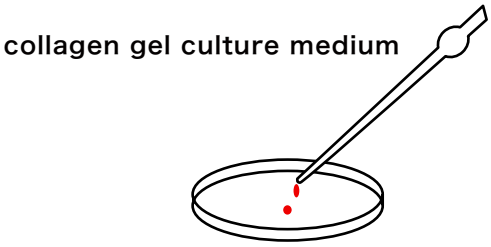
3) If necessary, add serum at 2 – 4˚C.

Note: Unused AteloCell™ can be kept for next use, but avoid repeated cycles of freezing and thawing. Subdivide the product into smaller reagent bottle and store in a freezer, and use only necessary amounts by thawing each time.

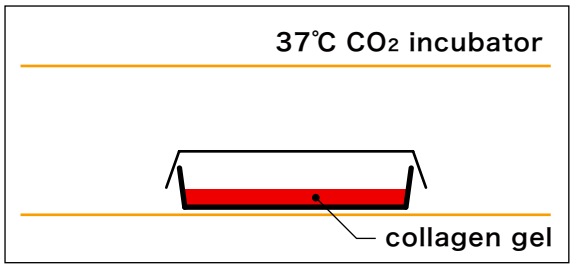
# III . Cell culture on collagen gels

## A. Culture on collagen gel medium

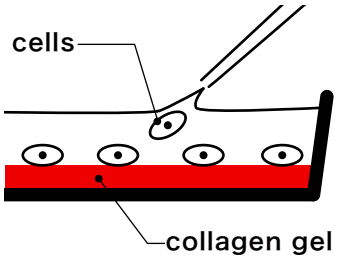
(1) Add an appropriate amount of collagen gel medium A or B to a culture dish (0.5 – 1 mL for a 35mm dish or 1 – 3 mL for a 50mm dish).

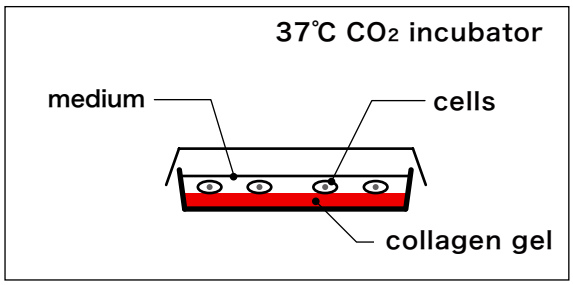



(2) Warm the culture dish at 37˚C in a $CO_2$ incubator for 30 min for gel formation.



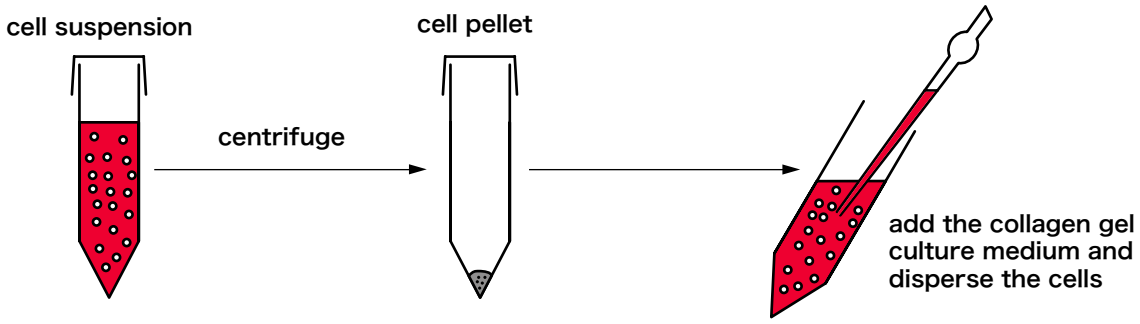

(3) Add cell suspension to start culture.



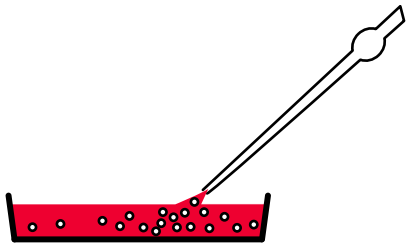



## Ⅳ . Collagen gel culture

(1) Centrifuge cell suspension and remove supernatant. Add collagen gel medium A or B to the cell pellet. Pipette to avoid air bubbles, then disperse cells.




(2) Add the mixture of collagen gel medium/cells to a culture plate.



(3) Incubate at 37˚C in a $CO_2$ incubator for gel formation and culture.