Sample

AteloCell®

Native collagen Bovine dermis 3mg/mL

Cat No.:	KOU-IAC-30	
Lot No.:	*	
ORIGIN:	Bovine dermis	
SOLUTION:	1 mM HCl	
STORAGE:	4°C	
REFERENCES:	Refer to the AteloCell® website http://www.cosmobio.com	
	Specification	Results
CONCENTRATION:	2.8～3.2 mg/mL	*
pH:	2.9～3.1	*
STERILITY TEST: (Medium: TGC-I and SCD)	Negative	Pass
CELL CULTURE TEST: (Cell: Human Fibroblast)	Normal	Pass
SPECIFIC OPTICAL ROTATION: $[\alpha]_D^{20}$	-370°～-430°	*
PURITY:	Total collagen > 95% by SDS-PAGE	Pass

FOR RESERCH USE ONLY, NOT FOR HUMAN BODY.

Manufactured by KOKEN Co., Ltd.

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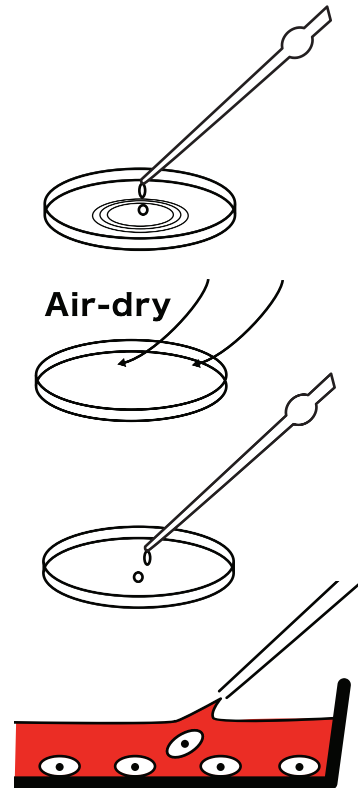
I . Coating cultureware with collagen



(1) Working on a clean bench, add an appropriate amount (1 – 2 mL for a 35 mm dish*1) of either 0.3%/0.5%*2 I-AC (Native collagen) or I-PC (Atelocollagen) to the cell cultureware and spread the solution over the entire surface.

(2) Keep the lid of the cell cultureware off and air-dry the cultureware below 25℃.

(3) Neutralize the surface of the cell cultureware by washing several times with PBS (150 mM NaCl, 20 mM Na_2HPO_4, pH 7.4).

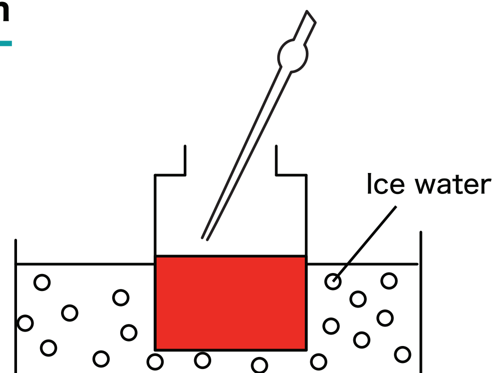
(4) Add the cell suspension and culture as usual.

*1: Due to the high viscosity of collagen solution, it will not spread over the whole cultureware surface when a small amount is applied.

*2: Dilute the collagen solution with distilled water adjusted to pH 3.0, if necessary.

II . Preparation of collagen-containing medium

A. Using I-AC (Native collagen) or I-PC (Atelocollagen)



Reagents

1. Medium (10 x concentration)*3 .... 1.0 mL
2. HEPES pH 7.4 (100 x concentration) .... 0.1 mL
3. NaHCO3 (100 x concentration) .... 0.1 mL
4. Distilled water .... 0.8 mL
5. I-AC (Native collagen) or I-PC (Atelocollagen) (either 0.3% or 0.5%) .... 8.0 mL

Reagent details

1. Medium (10 x concentration: 10 times concentration of cell culture medium prepared using powdered medium)*4 .... 1.0mL
2. HEPES pH 7.4 (f.c. 10 mM: 10 mM x 100 = 1000 mM 0.1mL
3. $NaHCO_3$ (f.c. 10 mM: 10 mM x 100 = 1000 mM 0.1mL
4. Distilled water 0.8mL
5. I-AC (Native collagen) or I-PC (Atelocollagen) (either 0.3% or 0.5%) 8.0mL

(1) Mix Reagents 1 through 4 while cooling in an ice water bath and then add the collagen solution. Since the collagen solution is viscous, pipette several times to remove the collagen attached to the pipette surface. Be careful not to form bubbles as it is difficult to remove them.

(2) If necessary, add serum to the mixture at 2 - 4℃.

*3: Although a 10 x concentration of Hanks' medium or Eagle's MEM, etc., can be easily prepared, it may be difficult to prepare a 10 x concentration of a rich medium such as DMEM. In such cases, prepare a 3-5 x concentration medium and adjust the amount of collagen solution and/or distilled water accordingly.

*4: The final collagen concentration will be approximately 0.24% and 0.4% when a 0.3% solution and 0.5% solution is used, respectively. In order to obtain a softer gel, reduce the amount of collagen solution and adjust the amount of water because the solidity of the collagen gel depends on the final collagen concentration. The minimum concentration of collagen required for gel formation is 0.1%.

B. Using atelocollagen neutral solutions

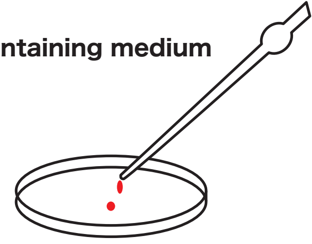
(1) Thaw the frozen atelocollagen neutral solution while stirring in a 25℃ warm water bath (without stirring, the solution may partially gel)*5.

(2) Keep the atelocollagen neutral solution in ice water after thawing.

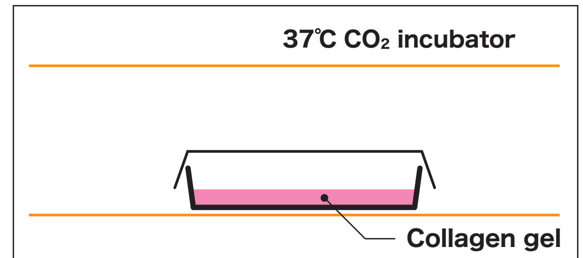
(3) If necessary, add serum to the solution at 2 - 4℃.

*5: Dispense the surplus solution into small vials and store in a freezer to avoid multiple freeze-thaw cycles.

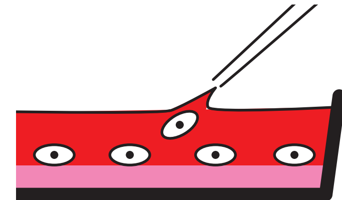
III . Cell culture on collagen gels



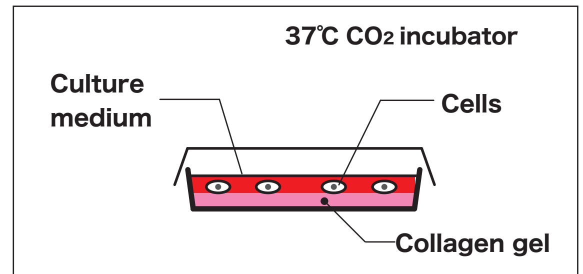
(1) Add an appropriate amount (0.5 - 1 mL for a 35 mm dish, or 1 - 3 mL for a 50 mm dish) of the collagen-containing medium described in IIA or IIB to the cell cultureware.



(2) Warm the solution in the cell cultureware at 37°C in a CO_2 incubator for 30 min to allow gel formation.

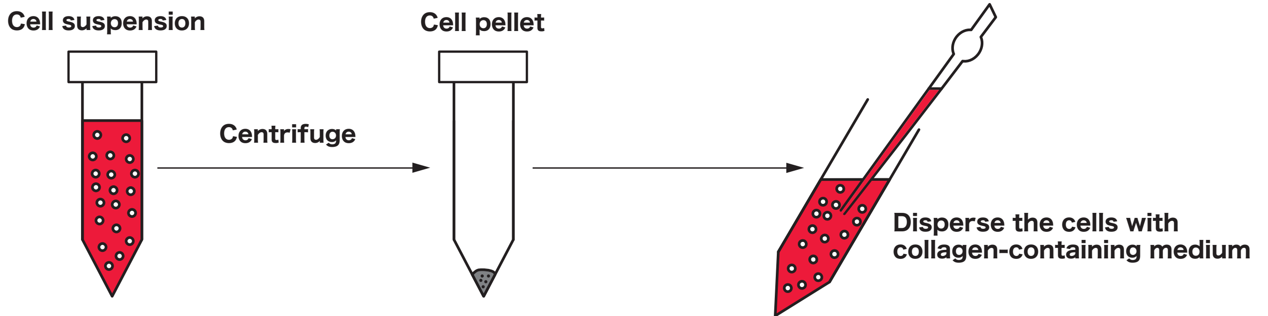


(3) Add the cell suspension onto the gel.

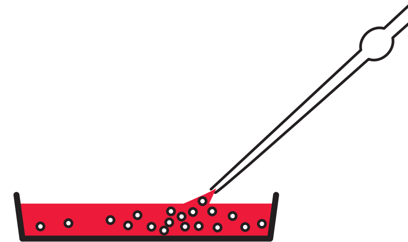


IV. Cell culture in collagen gels

(1) Centrifuge the cell suspension and remove the supernatant. Add the collagen-containing medium described in IIA or IIB to the cell pellet and disperse the cells. Be careful not to form bubbles because it is difficult to remove them.

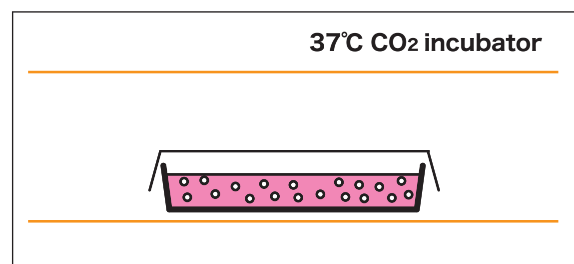


(2) Add the mixture of cells and collagen-containing medium to the cell cultureware.



(3) Warm the mixture in the cell cultureware at 37℃ in a CO_2 incubator to allow gel formation.

*6: The higher the concentration of collagen, the faster the gel will form. To prevent the cells from sinking during gel formation, keep the collagen concentration at 0.4% or higher.



(4) Add the cell culture medium onto the gel. Replace the medium as needed.