Instructions for Using Collagen Acidic Solution

Storage

Refrigerated (2-10° C)

Collagen Coating Method

Required Reagents

① Collagen Acidic Solution I-AC or I-PC (either 0.3% solution or 0.5% solution of each product can be used)

② 1mM HCl

③ PBS (-)

Procedure

(1) Dilute the collagen acidic solution approximately 10-fold on ice using chilled 1mM HCl. *1, 2

*1: Although the collagen concentration needs to be optimized according to the surface structure of the culture vessel and the adhesion capacity of the cells used, our research indicates that diluted solutions of 0.001-0.1% are commonly used.
*2: Since the collagen solution is viscous, mix slowly using a stirrer or rotator to avoid foaming.

(2) Add an appropriate amount of diluted collagen solution to the culture surface of the cultureware and spread it evenly. *3

*3: Due to its high viscosity, using too small a volume of collagen solution prevents complete coverage of the culture surface.

Reference volume:
35mm dish → approximately 1-2 mL
100mm dish → approximately 3-4 mL

(3) After allowing to stand at room temperature for at least 1 hour, remove the collagen solution from the cultureware. *4

*4: Depending on the cells, it may be more suitable to allow complete drying by leaving overnight.

(4) Wash several times with PBS to neutralize.
(5) Add the cell suspension and perform culture.

Preparation for Culture in Collagen Gel (Preparation Method for Collagen-Containing Medium)

Caution

Work on ice to avoid collagen denaturation and uneven gelation. Since Collagen Acidic Solution I-AC is prone to gelation, chill the tubes and reagents on ice or in a cold room.

Required reagents

(When preparing 10 mL)

1.	**10x concentrated medium** *1, 2	1mL (final concentration 1x)
2.	1M HEPES pH7.4	0.1mL (final concentration 10mM)
3.	1M $NaHCO_3$	0.1mL (final concentration 10mM)
4.	Distilled water	0.8mL
5.	Collagen Acidic Solution I-AC or I-PC *3, 4 (either 0.3% solution or 0.5% solution of each product can be used)	8mL

*1: Hanks' medium, Eagle's MEM, etc. can be prepared at 10x concentration, but "rich" media such as DMEM may not may not completely dissolve. In such cases, prepare at 3-5x concentration and adjust the amount of collagen or distilled water so that the final medium concentration is 1x.

*2: If you want to prepare a collagen gel without medium components, concentrated PBS can be used as a substitute.

*3: With this method, the final collagen concentration will be approximately 0.4% when using the 0.5% solution, and approximately 0.24% when using the 0.3% solution. Since the stiffness of the collagen gel depends on the collagen concentration, to prepare a softer gel, reduce the amount of the collagen solution (from step 5) and replace the difference with distilled water (gelation occurs down to a final collagen concentration of 0.1%).

*4: When preparing a gel from Collagen Acidic Solution I-PC 0.3% solution, there is concern that cells may sink during gel formation, so the use of 0.5% solution is recommended. However, the acidic collagen solution I-AC exhibits a rapid gelation rate and is an exception to this.

Procedure

(1) Add required reagents in the order of 1, 2, 3, 4 on ice, and 5 last. *5

*5: Since the collagen solution is highly viscous and the amount adhering to the vessel walls cannot be ignored, rinsing by pipetting is necessary. When pipetting, be careful not to introduce air bubbles into the solution, as they are difficult to remove once introduced.

(2) If serum is required, add it to this mixture at 2-4℃. *6

*6: Since serum may affect the gelation of collagen, it is recommended to form the gel without serum. The inside of the gel will be rapidly equilibrated by the liquid medium containing serum added after gelation.

Culture in Collagen Gel

Required Reagents

① Collagen-containing medium (refer to the previous section for method of preparation)

Procedure

(1) Harvest cells cultured in dishes or multi-well plates by enzymatic treatment, etc., and count the number of cells in the cell suspension.

(2) Aliquot the required number of cells, centrifuge, and prepare a cell pellet or cell suspension. *1, 2

*1: Although it depends on the purpose and duration of the experiment, it is recommended to optimize conditions starting from approximately 1.0-3.0 x 104 cells/mL.

*2: When using a cell suspension, 1/10 or less of the collagen-containing medium volume is desirable, as dilution will reduce the collagen concentration.

(3) Add the collagen-containing medium and mix well by pipetting.

(4) Dispense into culture vessels such as multi-well plates (*3), and quickly place in an incubator set at 37° C.

*3: The dispensing volume should be approximately half the amount of medium used for monolayer culture (50-100 µL for a 96-well plate)

(5) 1-2 hours, depending on the product, confirm gel formation (*4), add liquid medium containing serum, and perform culture. *5

*4: Collagen-containing medium prepared from I-AC generally gels in about 30 minutes, but incubation for 1 hour is recommended, and collagen-containing medium prepared from I-PC generally gels in about 1 hour, but incubation for 2 hours is recommended.

*5: Add approximately the same amount of liquid medium as used for monolayer culture (100-200 µL for a 96-well plate).

As culture progresses, the cell number increases compared to monolayer culture, so frequent medium changes are required.

RNA Recovery from Collagen Gel

Required Reagents

① Commercially available RNA extraction reagents or kits *1, 2, 3

*1: In our studies, phenol-based RNA extraction reagents tended to yield higher RNA amounts, while column-type kits tended to yield higher RNA purity.

*2: With column-type kits, collagen fibers derived from the collagen gel may clog the column, resulting in lower yields. In such cases, RNA yields may be stabilized by extracting with a phenol-based extraction solution, separating with chloroform, and then purifying RNA from the recovered aqueous phase following the instructions of the column-type kit.

*3: Since DNase treatment may reduce RNA yield determine its necessity according to the purpose of the experiment.

Procedure

(1) Remove the liquid medium from the collagen gel.

(2) Use commercially available RNA extraction reagents or kits considering the amount of collagen gel.

Cell collection

Required Reagent

① 0.2% Collagenase solution *1, 2

② PBS

*1: Collagenase for cell dispersion is recommended. For conditions or use of other types, refer to the instruction manuals of the product.

*2: To prepare the collagenase solution, use buffer containing 2mM Ca^{2+}, as it is required for collagenase activity.

Procedure

(1) Remove the liquid medium from the collagen gel.

(2) Gently break up the gel with the tip of the pipette tip.

(3) Add collagenase solution to a final concentration of 0.02%-0.2%.

Example: When 100 µL gel is prepared in a 96-well plate, add 100 µL of 0.2% collagenase solution (final concentration 0.1%).

(4) Incubateat 37℃.

(Dissolution time varies depending on the type and lot of collagenase, but dissolution can be achieved in approximately 15 minutes.)

(5) After confirming that the gel has dissolved, transfer the solution to a tube.

(6) Centrifuge and remove the supernatant.

(7) Add PBS as appropriate and resuspend. Centrifuge again and remove the supernatant.

(8) Repeat step (7) 3-5 times to remove collagenase.

Histological Evaluation

Required Equipment and Reagents

① 4% Paraformaldehyde (PFA)
② 30% Sucrose solution (in phosphate buffer, pH 7.4)
③ Embedding medium for frozen section preparation (e.g., Leica FSC22, SECTION-LAB SCEM)
④ Frozen section preparation device (cryostat)
⑤ Glass slides *1
⑥ Tris Buffered Saline containing 0.05% Tween20 (TBST)
⑦ Blocking agent or serum
⑧ Various primary antibodies and secondary antibodies
⑨ Nuclear staining solution (e.g., DAPI diluted in PBS)
⑩ PBS

*1: Since sections prepared during staining tend to peel off from the glass slides, it is recommended to prepare sections at 20 µm thickness or less and use highly adhesive glass slides with anti-peeling treatment.

Histological Evaluation （Continued）

Ⅰ. Frozen Section Preparation

(1) Remove the liquid medium from the collagen gel.

(2) Add 4% PFA and fix overnight at 4℃ protected from light.

(3) Remove the collagen gel from the culture vessel using a spatula or similar tool and transfer to 30% sucrose solution. *1

*1: Use sufficient amount of sucrose solution relative to the collagen gel to prevent ice crystal formation in the sample during freezing.

(4) Mix the collagen gel and sucrose solution at 4℃ using a rotator or similar device until the collagen gel sinks. *2

*2: Replace the sucrose solution as needed to thoroughly replace the water in the gel with sucrose solution.

(5) Remove the collagen gel, lightly blot off the sucrose solution with Kimwipes or similar, place in embedding medium for frozen section preparation, and allow to equilibrate for 1 hour.

(6) Place the gel in fresh embedding medium, rapidly freeze on dry ice, and store at -80° C if frozen sections are not prepared immediately.

(7) Prepare frozen sections using a cryostat. *3

*3: In our laboratory, sections are prepared with the cryostat chamber and specimen stage set at -35℃.

Ⅱ. Immunohistochemistry on Tissue Sections and Downstream Applications

(1) Wash glass slides with attached sections with PBS, then incubate twice for 5 minutes with fresh PBS. *4

*4: Since collagen gel sections tend to peel off from the glass slides, it is recommended to avoid using a shaker during washing, which is a common practice. If non-specific signals due to insufficiently washed antibodies are observed, increase the number of incubations or the incubation time.

(2) Perform permeabilization with TBST for 15 minutes.

(3) Block for 1 hour in a humidity chamber using blocking agent or serum.

(4) Dilute the primary antibody in TBST containing blocking agent or serum and incubate at 4℃ or room temperature.

(5) After washing with TBST, incubate twice for 5 minutes with fresh TBST. *4

(6) If performing co-staining, repeat steps (4)-(5).

(7) Dilute the secondary antibody mixture with TBST containing blocking agent or serum and incubate at 4℃ or room temperature.

(8) After washing with TBST, replace with fresh TBST and incubate for 5 minutes twice. *4

(9) After washing with PBS, perform nuclear staining.

(10) After washing with PBS, replace with fresh PBS and incubate for 5 minutes twice. *4

(11) Mount with mounting medium containing anti-fade reagent.

Collagen Acidic Solution

Catalog Number	Product		Size
KOU-IPC-30	Atelocollagen, Bovine dermis (Collagen Acidic Solution I-PC 3 mg/mL)	pH 3.0 Sterile	50mL/bottle
KOU-IPC-50	Atelocollagen, Bovine dermis (Collagen Acidic Solution I-PC 5 mg/mL)	pH 3.0 Sterile	50mL/bottle
KOU-IAC-30	Native collagen, Bovine dermis, (Collagen Acidic Solution I-AC 3 mg/mL)	pH 3.0 Sterile	50mL/bottle
KOU-IAC-50	Native collagen, Bovine dermis, (Collagen Acidic Solution I-AC 5 mg/mL)	pH 3.0 Sterile	50mL/bottle

Type of Collagen

Collagen Acidic Solution I-PC: Bovine dermal atelocollagen

Collagen Acidic Solution I-AC: Bovine dermal native collagen

Frequently Answered Questions

https://atelocollagen.com/en/atelocell/faq/01

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