Sample

COSMO BIO USA.

KOKEN

AteloCell®

Certificate of Analysis

FibColl® Atelocollagen Inserts 24　Cat. No. FAI-24
Lot No. ******

COLLAGEN ORIGIN:	Bovine dermis
COLLAGEN TYPE:	Type I atelocollagen
FORM:	Attached to a polystyrene frame
SIZE:	φ6.4 mm x approximately 35 μm
SURFACE AREA:	0.33 cm^2
STORAGE:	Room temperature
PRECAUTIONS:	- Avoid moisture during storage. - Refrain from re-drying once the membrane gets wet. These affect the permeability of the membrane. - Make sure the membrane is equilibrated with a medium before use.

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
CELL CULTURE TEST: (Cell: Human Fibroblast)	Normal	Pass

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

atelocollagen.com

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Storage conditions

Store in an aluminum bag at room temperature.*1

*1 Re-drying after humidification alters the collagen fiber structure and reduces permeability. Therefore, avoid storage in humid conditions.

Recommended 24 well plates

Inserts are optimized for use with Tissue Culture Multiwell Plates (IWAKI Product number: 3820-024). *2

*2 However, they may also be used with 24 well plates commercially available from various companies.

Cell seeding procedure

(1) Add 600 μL of media to wells and 100 μL of media to inserts and then allow inserts to equilibrate in a CO_2 incubator for at least 30 minutes.

(2) Remove media from plate wells and inserts. *3,4

*3 Pasteur pipettes can be used to remove media from wells while, to prevent membrane breakage, micropipettes arerecommended for media removal from inserts.

*4 Inserts are designed with two openings to permit convenient media exchange from wells without the need for insert removal.

(3) Add 600 μL fresh media to wells. *5

*5 For co-culture experiments, add 600 μL of the first type of of cell suspension to wells.

(4) Add 100 μL of cell suspension to inserts. As a guide, the optimal number of fibroblasts to be seeded on insert membranes (culture area 0.33 cm^2) is 1 x 10^4 cells*6.

*6 However, the optimal number of seeded cells depends on cell type and should be determined by the user in pilot experiments.

(5) Culture in a CO_2 incubator.

Changing Medium

(1) Remove media from wells and inserts.*7,8

*7 Pasteur pipettes can be used to remove media from wells while, to prevent membrane breakage,micropipettes are recommended for media removal from inserts.

*8 Inserts are designed with two openings to permit convenient media exchange from wells without the need for insert removal.

(2) Add 100 μLof cell suspension to inserts.

(3) Add 600 μL fresh media to wells.

(4) Culture in a CO_2 incubator.

Cell Collection

Preparation

①PBS (-)

②Trypsin solution (0.05% trypsin/EDTA etc.)

Procedure

(1) Remove media from wells and inserts.

(2) Wash with a calcium-free buffer(for example PBS (-)) and then remove buffer.

(3) Add 100μL Trypsin solution and incubate for about ~2 minutes.*9

*9 Pre-determine the appropriate enzyme type and treatment time according to the cell type.

(4) Collect cells by pipetting with an appropriate buffer solution.

Counting live cells

Use a commercially available live cell count measurement kit (WST-8, etc.).

RNA extraction

Preparation

①PBS (-)

②Commercial RNA extraction reagent or kit

Procedure

(1) After removing media, wash with 100μL of PBS (-) to inserts and 600μL to wells and remove.

(2) Repeat step (1).

(3) Use a commercially available RNA extraction reagent/kit according to the manufacturer's instructions.

Phase-contrast / Fluorescence microscopy

* 10 Although insert membranes are not as clear as culture vessel plastic, it is possible to observe cells using phase contrast microscopy.

* 11 Fluorescence observation during cell culture is difficult due to blurring from the influence of the membrane. However, fluorescence microscopy can be used after removing membranes from insert frames by the method described below.

Removal of collagen membranes from insert frames for cell observation

Required equipment and reagents

① Fixative (4% paraformaldehyde, etc.)
② PBS
③ Tweezers
④ Commercially available disposable scalpel (such as KAI Disposable Scalpel No.11)
⑤ Glass slides and coverslips
⑥ Mounting medium

Procedure

(1) Remove culture medium, wash with PBS, fix with appropriate fixative, and wash with PBS after removing fixative.

(2) Remove inserts using tweezers and place inverted on a stable surface (plate lid, for example).

(3) Use a commercially available disposable scalpel to carefully cut along the periphery of the collagen membrane using the inside of the insert frame as a guide (Fig. 1).*12,13

*12 Move the scalpel in small up and down motions to cut along the insert frame.
*13 We recommend blades with narrow pointed tips, such as No.11 Disposable Scalpel (KaiCorporation) (Fig. 2).

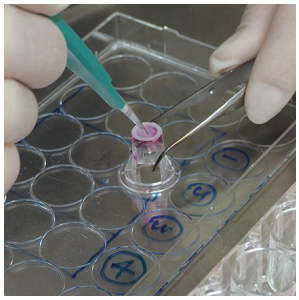
Fig1. Excising membranes


Fig2. Blade shapes suitable for excising membranes

(4) Collect collagen membranes with tweezers (Fig. 3).

(5) When observing cells, place each collagen membrane on a glass slide, add PBS or mounting reagent (Fig. 4).*14

*14 Embed and observe collagen membranes with the cell-containing side facing up toward the microscope lens.

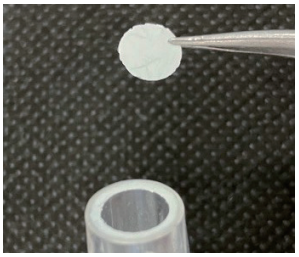
Fig3. Excised collagen membrane

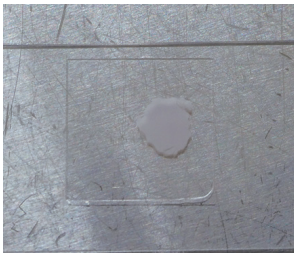
Fig4. Collagen membrane mounted under a coverslip

Section preparation

Frozen sections can be made in the same way as tissue sections, after the collagen membrane was removed from the frame.
The adhesive film (Kawamoto) method may be effective for preparation of frozen sections from collagen membranes that have been excised from insert frames as described above.

Histological evaluation

Required equipment and reagents

① Fixative (4% paraformaldehyde, etc.)
② TBS-T (Tris Buffered Saline + 0.05% Tween20)
③ Commercially available blocking agent, etc.
④ PBS
⑤ Various primary antibodies, secondary antibodies
⑥ Nuclear staining solution*15

*15 Collagen itself has blue autofluorescence, so a nuclear stain such as DRAQ5 is preferable over DAPI.

Procedure

(1) Follow the procedure described above to fix and remove collagen membranes from insert frames.

(2) Shape excised collagen membranes as shown in Figure 5 to introduce asymmetry allowing discrimination between front and back sides.

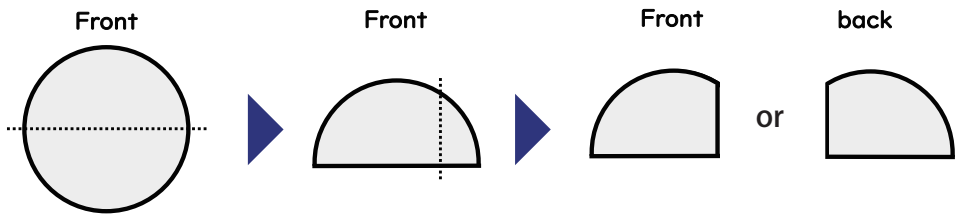

Fig5. Shaping of excised collagen membrane

(3) Add TBS-T to shaped membranes and incubate at room temperature for 15 minutes.

(4) Remove TBS-T, add blocking agent, and incubate at room temperature for 1 hour.

(5) Add primary antibody solution diluted in TBS-T containing blocking agent and incubate overnight at 4° C.

(6) Remove the primary antibody solution and wash with TBS-T three times.

(7) Add the secondary antibody solution diluted in TBS-T containing blocking agent and incubate at room temperature for 3 hours in the dark.

(8) Remove the secondary antibody solution, wash with TBS-T three times, and replace with PBS.

(9) Add a nuclear staining solution diluted with PBS and incubate at room temperature for 15 minutes in the dark.

(10) Remove the nuclear staining solution and wash once with PBS.

(11) After mounting on a glass slide with mounting medium, observe with the cell-containing side facing up toward the microscope lens.