COSMO BIO CO., LTD.
Inspiration for Life Science

KOKEN

# AteloCell®

## DATA SHEET

**3D Ready Atelocollagen DMEM-LG1 Cat No. 3D-LG01**
**3D Ready Atelocollagen DMEM-LG5 Cat No. 3D-LG05**
Lot No. ******

| | |
|---|---|
| COLLAGEN ORIGIN: | Bovine dermis |
| COLLAGEN TYPE: | Type I Atelocollagen |
| CELL CULTURE MEDIUM: | DMEM Low Glucose (1,000 mg/L glucose) |
| STORAGE: | -20°C |
| PRECAUTIONS: | - Thaw the product in a refrigerator overnight.<br>It is stable up to one month in the refrigerator.<br>- Refrain from multiple freeze-thaw cycles.<br>It is advised to dispense and freeze once defrosted, if necessary.<br>- Implement measures to keep it cool to avoid unnecessary fibril formation. |

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

**English instruction manuals are available through your local distributors.**

| | Specification | Results |
|---|---|---|
| COLLAGEN CONCENTRATION: | 3.6～4.4 mg/mL | *.* mg/mL |
| pH: | 7.1～7.7 | *.* |
| STERILITY TEST:<br>(Medium: TGC-I and SCD) | Negative | Pass |
| CELL CULTURE TEST:<br>(Cell: Human Fibroblast) | Normal | Pass |

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


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# AteloCell®

## DATA SHEET

**3D Ready Atelocollagen DMEM-HG1 Cat No. 3D-HG01**
**3D Ready Atelocollagen DMEM-HG5 Cat No. 3D-HG05**
Lot No. ******

| | |
|---|---|
| COLLAGEN ORIGIN: | Bovine dermis |
| COLLAGEN TYPE: | Type I Atelocollagen |
| CELL CULTURE MEDIUM: | DMEM High Glucose (4,500 mg/L glucose) |
| STORAGE: | -20°C |
| PRECAUTIONS: | - Thaw the product in a refrigerator overnight.<br>It is stable up to one month in the refrigerator.<br>- Refrain from multiple freeze-thaw cycles.<br>It is advised to dispense and freeze once defrosted, if necessary.<br>- Implement measures to keep it cool to avoid unnecessary fibril formation. |

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# Instructions for using 3D Ready Atelocollagen

## Ⅰ. Handling of 3D Ready Atelocollagen

(1) Upon receipt of 3D Ready Atelocollagen, store it in a freezer. Before use, thaw the frozen product in a refrigerator overnight.

*1: After thawing, reagents may be stored for up to 1 month at 2-8℃ before opening. Once you have opened the reagent, you do not need to use it immediately. It can be stored at 2-8℃ for several weeks after it has been opened, if it will be used soon. For longer storage, it should be kept at -20℃.

*2: You can aliquot the 3D Ready Atelocollagen when thawing for the first time and store the aliquots in a freezer. Avoid repeated freeze-thaw cycles.

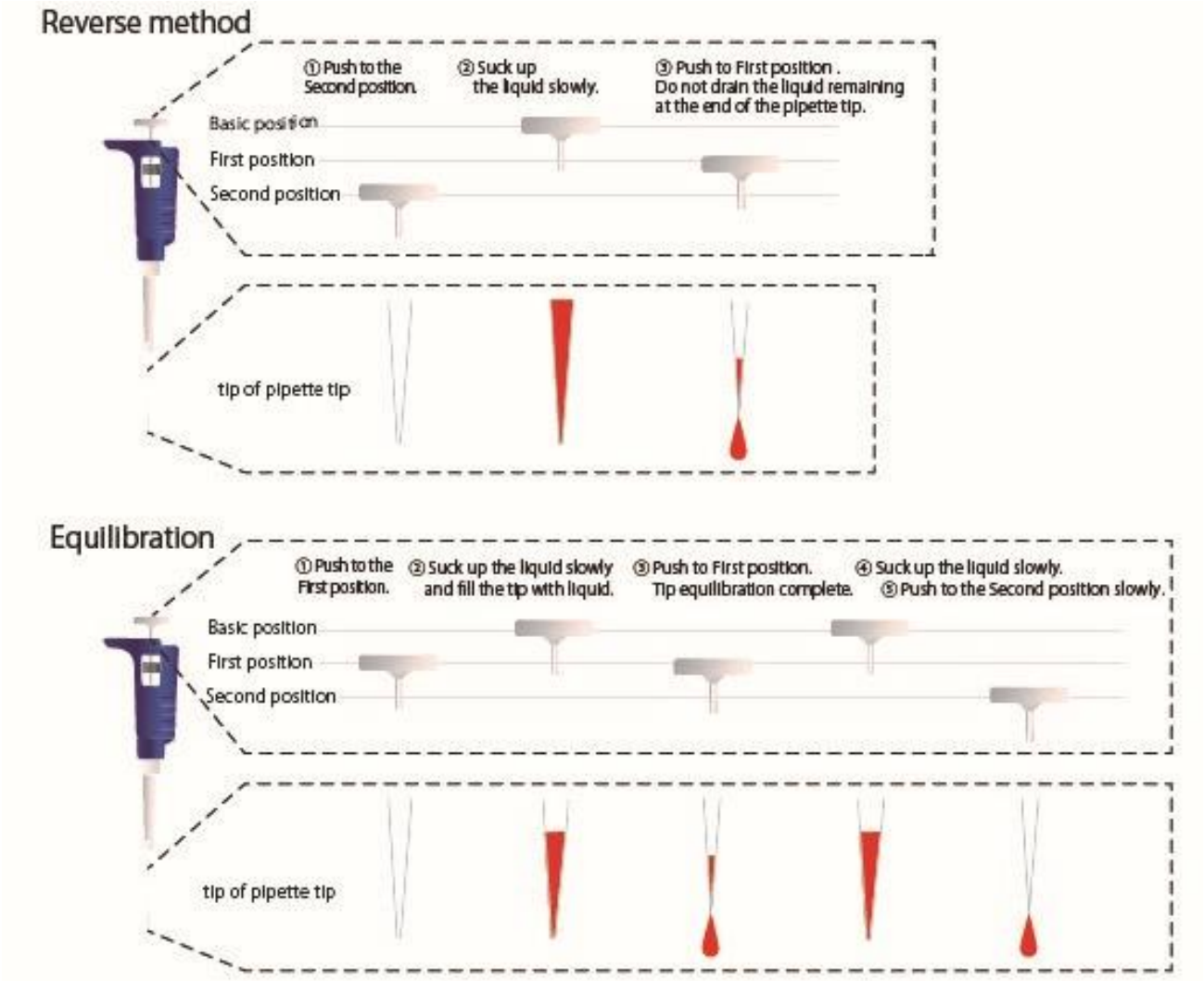
(2) Keep the products cool at all times by placing on ice when being dispensed.

*3: Pay attention to temperature control. If the liquid temperature rises, the collagen contained in the product will fibrillates and gel.

(3) The product is highly viscous and will adhere to the inside of the pipette tip, causing a loss of liquid volume. To reduce loss, pipette slowly using the reverse method shown below and wait a few seconds after the solution has been dispensed.

*4: If you use a tip with a filter, equilibration is recommended instead of the reverse method because a large set volume may cause the solution to adhere the filter inside the tip.

# Instructions for using 3D Ready Atelocollagen




## Ⅱ. Culturing in collagen gel

(1) Collect cells cultured in a dish or multi-well plate by enzyme treatment and count the number of cells in the cell suspension.

(2) Collect the required number of cells and centrifuge to prepare a cell pellet or cell suspension.

*1: Although it depends on the purpose and period of the experiment, if you are using a cell pellet, it is generally recommended to use a cell of 1.0 to 3.0 × $10^4$ cells/mL.

*2: If you use a cell suspension, it is diluted and the collagen concentration will have decreased, so it is better to use less than 1/10 the amount of the 3D Ready Atelocollagen.

(3) Add the 3D Ready Atelocollagen and mix well by pipetting.

(4) Dispense into a culture container such as a multi-well plate and immediately place it in a 37℃ incubator.

*3: The amount to be dispensed should be about half the volume of the medium typically used for flat culture (Ex. 50-100 µL for 96-well plate).

(5) After 2 hours, confirm that a gel is formed, then add a serum-containing medium to culture.

*4: The product generally gels in about 1 hour, but the collagen gel surface may collapse when adding medium, so it is recommended to incubate for 2 hours before adding serum-containing medium.

*5: Add approximately the same amount of medium as that used for flat culture (Ex.100-200 µL in a 96-well plate). As the culture progresses, the number of cells will increase compared to the flat culture, and thus frequent medium replacement is required.

*6: We recommend not using serum when forming gels, as serum can affect the gelling of collagen. The inside of the gel is quickly equilibrated by the serum-containing medium added after gelation (May happen within 10 seconds up to a couple of minutes).

## Ⅲ. RNA recovery from collagen gel

(1) Remove the medium on the collagen gel.

(2) Use a commercially available RNA extraction reagent or kit that is appropriate for the amount of collagen gel,

*1: Our studies confirmed that the RNA yield was high when using a phenol-based RNA extraction reagent. We also found that the RNA purity was high when using a column type kit.

## Ⅳ. Cell recovery from collagen gel

| | | |
|---|---|---|
| 1. | Collagenase solution 1～2% | Appropriate amount |
| 2. | PBS | Appropriate amount |

*1: Collagenase for cell dispersion is recommended. For other types of collagenase, see the protocol of each reagent manufacturer.

*2: Use the buffer that prepares the collagenase solution that contains 2mM $Ca^{2+}$ required for the activity of the collagenase.

**Procedure**

(1) Remove the medium on the collagen gel.

(2) Lightly crush the gel with the tip of the tip.

(3) Add Collagenase solution so that the final concentration is between 0.02% and 0.2%. The concentration depends on cell-type.
Ex.) 100µl of 0.2% collagenase solution is added (final concentration 0.1%) when 100uL of gel is produced in a 96well plate.

(4) Shake at 37℃ for 30 minutes to 1 hour.

(5) After confirming that the gel has dissolved, transfer the dissolution medium to the tube.

(6) Centrifuge to remove the supernatant.

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

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

## Ⅴ. Evaluation method after incubation

Since there are no precautions that are unique to collagen, it is possible to use a general evaluation methods.

(For reference, frozen sections and immunohistochemistry are described below.)

## Ⅵ. Frozen section preparation from collagen gel

(1) Remove the medium from on top of the collagen gel.

(2) To fix, add 4% PFA and let stand overnight at 4℃ covered and protected from light.

(3) Remove the collagen gel from the culture vessel with a spoon and transfer it to a 30% (w/v) Sucrose solution (in phosphate buffer, pH 7.4).

*1: Use sufficient sucrose solution (approximately > 10 times volume of collagen gel) for the collagen gel to prevent ice crystals from forming in the sample during freezing.

(4) Place the collagen gel and sucrose solution on a rotator at 4 degrees and mix slowly until the collagen gel sinks. Mix slowly to avoid bubble formation and reduce risk of breaking the gel.

*2: To facilitate the gel sinking, replace the sucrose solution as necessary.

(5) Remove the collagen gel from sucrose solution and remove the sucrose solution on the gel by dabbing lightly with a Kimwipe. Put it in an embedding agent for frozen section preparation (O.C.T. compound, etc.), and let it acclimatize for 1 hour.

(6) Place the gel in a new embedding agent for frozen section preparation and freeze it rapidly on dry ice. If you do not prepare frozen sections immediately, store at -80°C.

(7) Prepare frozen section using a cryostat.

*3: Since the sections prepared at the time of staining are easily peeled off from the slide glass, prepare sections with a thickness of 20 μm or less. It is recommended to use highly adhesive glass slides with anti-peeling treatment.

(8) Place the prepared sections in a container containing silica gel and store at 4°C protected from light.

## Ⅶ. Immunohistochemical staining after section preparation

(1) After washing the glass slide with the attached section with PBS, change PBS and incubate twice for 5 minutes (protected from light, room temperature).

*1: It is recommended to avoid the use of shakers, which is a common method, because the collagen gel slices are easily peeled off from the slide glass. If a non-specific signal due to unwashed antibody is seen, increase the number of incubations or time.

(2) Permeabilize with TBST (Tris Buffered Saline + 0.05% Tween 20) for 15 minutes.

(3) Add blocking agent or serum for 1 hour in a moist chamber.

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

(5) After washing with TBST, change TBST and incubate twice for 5 minutes.

*2: Same as *1.

(6) When co-staining, repeat (4) ～ (5).

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

(8) After washing with TBST, change TBST and incubate twice for 5 minutes.

*3: Same as *1.

(9) After washing with PBS, stain the nucleus with DAPI etc. diluted with PBS.

(10) After washing with PBS, change PBS and incubate twice for 5 minutes.

*4: Same as *1.

(11) Apply a mountant containing anti-fade agent.