Sample

KOKEN
AteloCell®

Collagen microspheres

Cat No.:	KOU-MIC-00
Lot No.:	*
ORIGIN:	Bovine dermis
STORAGE:	4°C (Do not freeze)
REFERENCES:	Refer to the AteloCell® website http://www.cosmobiousa.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.

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Microsphere storage

2～10℃ 【Do not freeze】

Microsphere handling

Microspheres tend to adhere to highly hydrophobic plastic tubes and tips, causing loss. Thus, it is best to use products engineered for low protein adsorption, coated with hydrophilic polymers. (For example: Eppendorf Protein LoBind series, etc.)

Preparation

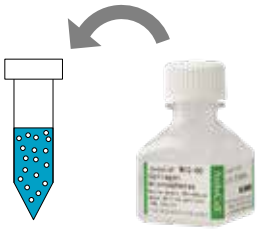
A.Microsphere preparation (replacing PBS carrier with user–selected medium) in a 50 mL centrifuge tube and transfer to a cell culture container

Required equipment and reagents

①50 mL centrifuge tube

②Medium

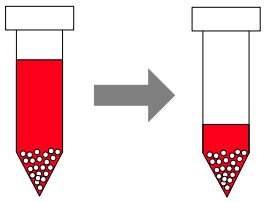
Procedure



(1)Swirl to resuspend microspheres and pour the entire contents of the product bottle*1 into a 50 mL centrifuge tube.

*1 The product bottle contains 15 mL of microspheres suspended in PBS to a volume of 25 mL.

(2)To recover microspheres remaining in the bottle, introduce approximately 8 mL of culture medium into the product bottle, swirl, and transfer to the centrifuge tube, repeating the process three times. After this step, the volume of fluid in the centrifuge tube will be ~49 mL.

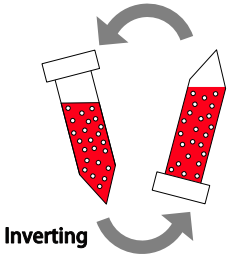


(3)Centrifuge at 25° C for 5 min at 500 x g*2,3 and carefully remove 30 mL of supernatant*4.

*2 Gravity sedimentation is effective as an alternative to centrifugation.

*3 As rapid centrifugal deceleration may unsettle microspheres, the centrifuge deceleration setting should be adjusted appropriately.

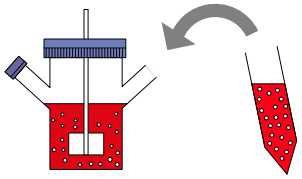
*4 To prevent microsphere loss from unsettling during supernatant removal, it is the best to leave behind at least 10 mL.



(4)Add 30 mL of culture medium and mix by gently inverting.

(5)Repeat steps (3)～(4) twice.

(6)Transfer microspheres newly suspended in ~49 mL of medium to a culture container such as a spinner flask or a bioreactor.



B.Microsphere preparation (replacing PBS carrier with user–selected medium) directly in a spinner flask

The following steps are for a 125 mL volume spinner flask (75 mL maximum working volume).

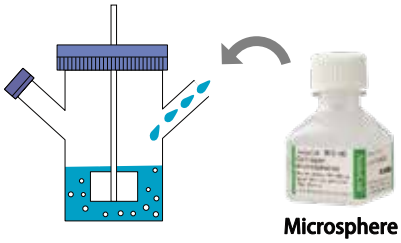
Required equipment and reagents

① Spinner flask*1 125 mL volume
② Medium

*1 Select a spinner flask type with arms that permit pipette access to media in the culture chamber.

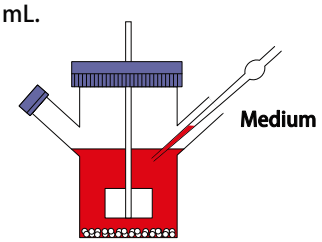
Procedure

(1)Pour the entire contents of the product bottle*2 into an appropriately shaped spinner flask*1.

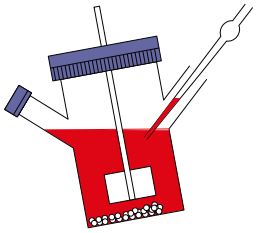


*2 The product bottle contains 15 mL of microspheres suspended in PBS to a volume of 25 mL.

(1)To recover microspheres remaining in the product bottle, introduce 25 mL of culture medium into the bottle, swirl, and transfer to the spinner flask, repeating the process three times. After this step, the volume of fluid in the spinner flask will be ~100 mL.

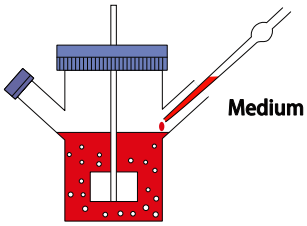


(2)Wait ~30 minutes for the microspheres to settle, and then carefully remove 50 mL of supernatant*3.



*3 The shape of the spinner flask arms will influence the ease of supernatant removal. To prevent microsphere loss from unsettling during supernatant removal, it is best to leave behind at least 30 mL.

(3)Add 30 mL of culture medium and swirl gently.



(4)Repeat steps (3)～(4) once, and then perform a final step (3)*4 such that the final volume of fluid in the spinner flask is ~50 mL.

*4 If microspheres are combined from more than one product (15 mL) , increase the repetitions of steps (3)～(4) to ensure the larger volume of microspheres is fully resuspend in medium.

Cell culture with microspheres using a spinner flask

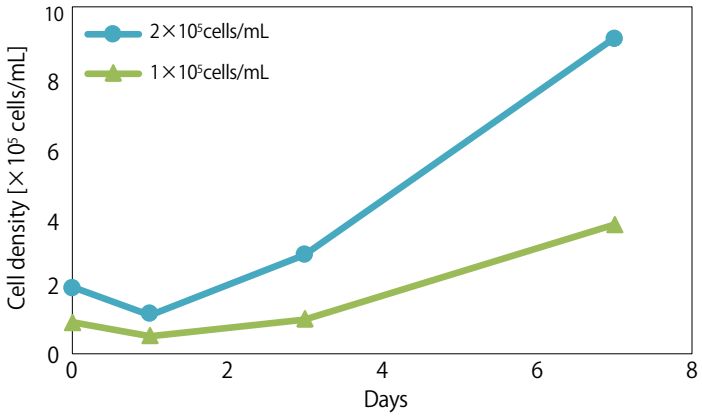
Required equipment and reagents

① Spinner flask
② Stirring and heating equipment

I.Seeding and expanding cells

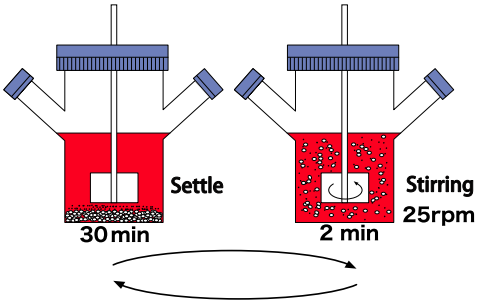
(1)Warm microsphere/medium suspension held in a spinner flask [prepared as described above (A or B)] to 37 ° C, add cell suspension and immediately stir (25 rpm, 1-2 min.) to evenly disperse microspheres and cells. Approximate number of cells to seed: 1 x 10^7 cells*1 per 50 mL medium (containing 15 mL microspheres)

*1 This number is based on using fibroblasts as an example. The optimal number of cells to seed will need to be adjusted according to cell type and experimental purpose. We observed fibroblasts seeded at two different densities to proliferate as shown in the figure below (in-house data).



(2)Wait ~30 minutes for microspheres to settle and then stir at 25 rpm for 2 minutes. Repeat this procedure 12 times (~6 hours total), and thereafter stir continuously at 25 rpm*2.

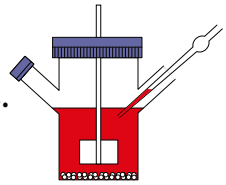
*2 As the cells proliferate in the flask, gradually and carefully increase the stirring rate beyond 25 rpm. The rotation speed should be chosen carefully because too fast rotation can cause cell damage, whereas too slow rotation inhibits cell proliferation due to formation of cellular bridges between microspheres.



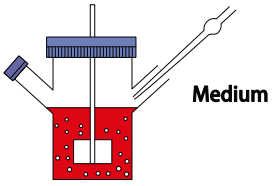
II . Medium change*3

(1) Stop stirring and allow the microspheres to settle about 30 minutes. Remove supernatant.

(2) Add fresh medium



*3 Change the medium 2–3 times a week. As the number of cells increases (monitor by pH color indicator in medium) increase the media change frequency.



Small–scale cell culture with microspheresin dishes

Required equipment and reagents

① 35mm cell culture dish with low adhesion surface

② Bench-top shaker (wave shaking type)

Procedure

(1) Transfer 300 μL of a uniformly resuspended microsphere suspension to a microtube, centrifuge and remove the supernatant.

(2) Add culture medium, centrifuge, and remove the supernatant.

(3) Suspend in 300 μL of medium and soak. Example: Soak overnight at 4° C.

(4) Combine 300 μL of medium–resuspended microspheres with 300 μL of cell suspension and transfer to a low adhesive surface 35mm cell culture dish and mix gently. Place in an incubator for30minutes.

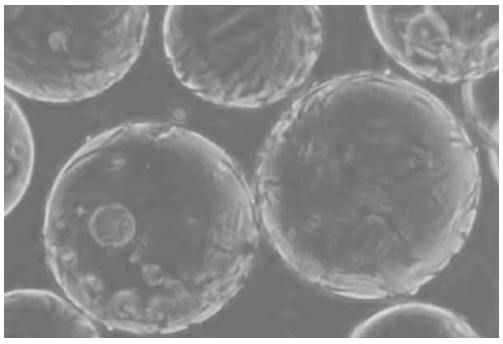
Approximate number of cells to seed: 1 x 10^5 cells / 300μL microsphere suspension

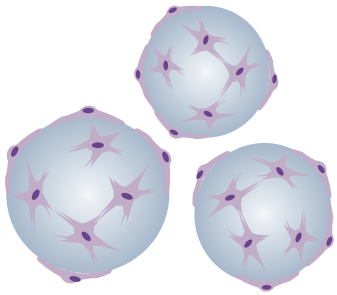
(5) Rock the dish for 2 minutes using a small tabletop rocker, then let it sit for about 30 minutes.
Approximate rocking conditions: Tilt angle 6 degrees, rotation speed 10 rpm

(6) Repeat step (5) 8 times to promote cell adherence to the microspheres.

(7) Add 1.5 mL of medium to the dish and incubate with rocking.
Approximate rocking conditions: Tilt angle 6 degrees, rotation speed 15 rpm

(8) For medium change, stop rocking, allow microspheres to settle, and replace half the supernatant volume.





Adherence of cells to the microsphere surface can be observed by phase contrast microscopy

Phase contrast micrograph (left) and diagrammatic depiction (right).

Harvesting cells from microspheres

A.Collagenase method

Reagents

① 0.2% collagenase solution*1

② PBS

*1 Collagenase is recommended for dissolution of microspheres and cell dispersal. Collagenase buffers should contain 2 mM Ca^{2+}, which is required for collagenase activity. For alternative cell dispersion solutions, refer to the instruction manual of the chosen product.

Procedure

(1) Transfer the culture medium containing cells and microspheres to a centrifuge tube.

(2) Add a volume of 0.2% collagenase solution equivalent to the culture volume so that the final concentration of collagenase is 0.1%, and incubate at 37° C.*2

*2 Microsphere dissolution time varies depending on the type and lot of collagenase, but typically requires 30 to 60 minutes.

(3) After confirming by phase contrast microscopy that microspheres have dissolved, centrifuge and remove the supernatant

(4) Add sufficient PBS to resuspend the cells, mix, centrifuge, and remove the supernatant.

(5) Repeat step (4) 3 to 5 times to remove collagenase.

B. Trypsin method*3

*3 We recommend the collagenase method for cell types with high adhesion to collagen as they are likely to be resistant to trypsin-mediated removal from microspheres.

Reagent

① PBS

② Trypsin solution (0.5% trypsin solution, etc.)

③ Cell strainer (pore size: 40 and 100 μm)

Procedure

(1) Collect the culture medium containing microspheres into a centrifuge tube, centrifuge, and remove the supernatant.

(2) Resuspend in PBS for washing, centrifuge and remove the supernatant.

(3) Resuspend in the trypsin solution and incubate for 15 min at 37 ° C.*4

*4 Enzyme solution type and treatment time should be adapted to chosen cell type.

(4) Add medium and resuspend microspheres and cells by pipeting up and down a few times.

(5) To remove microspheres and collect cells successively filter enzyme-treated cell suspension through cell strainers of different pore size: first 100 μm and then 40 μm.

RNA recovery

Reagents

① Commercially available RNA extraction reagent or kit*1

*1 There are examples of RNA recovery using phenol extraction reagents and spin column methods.

Procedure

(1) Collect the culture medium containing cells and microspheres into a centrifuge tube.

(2) Centrifuge and remove the supernatant.

(3) Harvest and purify RNA according to the instruction manual of the chosen RNA extraction product.

Histological observation of cells on microspheres

Reagents

① 4% paraformaldehyde (PFA)

② 0.2%TritonX-100

③ Bovine serum albumin（BSA）

④ PBS

⑤ Optional primary and secondary antibodies

⑥ Nuclear staining solution*1

*1 Since collagen exhibits blue autofluorescence, it is easier to observe using a nuclear staining solution such as DRAQ5 instead of DAPI.

Procedure

(1) Collect the culture medium containing microsphere-adherent cells into a centrifuge tube.

(2) Centrifuge and remove the supernatant.

(3) Fix the microsphere-adherent cells in 4% PFA.

(4) Wash with PBS and then permeabilize with 0.2% TritonX-100.

(5) Add primary antibody in 0.1% BSA and incubate at 4° C for 16 hours.

(6) Wash with PBS, add a secondary antibody solution and incubate for 1 hour at room temperature.

(7) Wash with PBS and perform nuclear staining.

Microspheres ordering information

Cat. No.	Description	Specification	Size
KOU-MIC-00	Atelocollagen Microspheres	Particle number : Approx. 3 million particles/15mL Surface area : Approx. 3,800 cm^2/15mL Size : φ100-400 μm	15 ml/bottle * *Total volume including solvent (PBS) is 25mL

Types of collagen

Atelocollagen derived from bovine skin

FAQ

https://www.cosmobiousa.com/products/collagen-microspheres

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