Cell passage protocol for collagen gel-cultured cells by Brighatse-C

The following is an example of an experiment in which human dermal fibroblasts ((NHDF (Normal Human Dermal Fibroblast)) were cultured on collagen gel for 1 week, then the collagen gel was digested with Brightase-C to collect the cells.
ASC：Acid soluble collagen
PSC：Pepsin-solubilized collagen

Experimental procedure

1. Gel Preparation and Cell Culture

① Prepare 1 mL of collagen gel per well in a 24-well plate using ASC or PSC solutions. Collagen gel concentration: 1 mg/mL
※For gel preparation, please refer to Reagent Collagen Gel Usage.
② Seed NHDF at 5×10^3 cells/well.
③ Incubate cells in 1 mL of 10 % FBS/DMEM in each well and change the medium every 3 days.

2. Cell Recovery

The amount of Brightase-C used for collagen gel digestion depends on the concentration and volume of collagen gel.

In case of ASC

① Dissolve Brightase-C (40 mg) in 2 mL of sterile water to make a 20 mg/mL solution.
② Dilute the stock solution of Brightase-C with 2 mM $CaCl_2$/ PBS prepare a 1 mg/mL solution. 2 mM $CaCl_2$/ PBS is prepared immediately before use and sterilized by 0.22 μm filter.
③ Remove medium.
④ Wash each well of gel culture 3 times with 1 mL of PBS.
⑤ After adding 1 mL of Brighatse-C solution (1 mg/mL) to each well, the wells are placed in a CO_2 incubator and incubated at 37℃ for 1 hour.
⑥ After confirming that all the collagen gel has dissolved, collect the solution from each well and place it in a 15 mL tube. If collagen gel remains, incubate the wells for an additional 30 minutes.

⑦ Centrifuge at 1500 rpm for 5 minutes.
⑧ Remove supernatant by aspirator.
⑨ Add 10 mL of PBS and lightly suspend the cells.
⑩ Repeat washing process (⑨～⑪) 5 times.
⑪ After centrifugation at 1500 rpm for 5 minutes, remove the supernatant using an aspirator.
⑫ Harvest cells.

In case of PSC

① Dissolve Brightase-C (40 mg) in 2 mL of sterile water to make a 20 mg/mL solution.
② Dilute the stock solution of Brightase-C with 2 mM $CaCl_2$/ PBS prepare a 100 μg/mL ~ 1 mg/mL solution. 2 mM $CaCl_2$/ PBS is prepared immediately before use and sterilized by 0.22 μm filter.
③ Remove medium.
④ Wash each well of gel culture 3 times with 1 mL of PBS.
⑤ After adding 1 mL of Brighatse-C solution (100 μg/mL ~ 1 mg/mL) to each well, the wells are placed in a CO_2 incubator and incubated at 37℃ for 30 minutes to 1 hour.
⑥ After confirming that all the collagen gel has dissolved, collect the solution from each well and place it in a 15 mL tube. If collagen gel remains, incubate the wells for an additional 30 minutes.
⑦ Centrifuge at 1500 rpm for 5 minutes.
⑧ Remove supernatant by aspirator.
⑨ Add 10 mL of PBS and gently suspend the cells.
⑩ Repeat washing process (⑨～⑪) 5 times.
⑪ After centrifugation at 1500 rpm for 5 minutes, remove the supernatant using an aspirator.
⑫ Harvest cells.

3. Cell passaging

① Add 2 mL of 0.05 % Trypsin/EDTA solution to the cell pellet and suspend. Incubate the cell suspension in a CO_2 incubator at 37℃ for 5 minutes.
② Centrifuge at 1500 rpm for 5 minutes.
③ Remove supernatant with aspirate.
④ Add 5 mL of PBS and gently suspend cells.
⑤ After centrifugation at 1500 rpm for 5 minutes, remove the supernatant with aspirate.

⑥ Resuspend cells in 10 % FBS/DMEM and seed cells into dishes.

⑦ In case of collagen gel culture, observe the wells the next day to confirm that the collagen gel has not been digested by the remaining Brightase-C.

Precautions

- Since more than 1 mM Ca^{2+} is required for the action of Brightase-C, Brightase-C diluted with 2 mM $CaCl_2$/PBS should be used for collagen gel digestion. Since long-term storage of 2 mM $CaCl_2$/PBS causes precipitation of calcium phosphate. Therefore, filter-sterilized 1 M $CaCl_2$ solution is added to PBS to prepare it immediately before use.
- The following 3 steps after collagen gel digestion with Brightase-C should be performed at least 5 times.
  1. Centrifugation of cell suspension
  2. Aspiration removal of supernatant
  3. Washing cell pellet with PBS

  Insufficient washing process may cause collagen gel to degrade due to the remaining Brightase-C when the cells are seeded in/on the collagen gel.
- If the cells are subcultured after recovery by Brighatse-C, Trypsin/EDTA treatment should be performed. The purpose of the Trypsin/EDTA treatment is to inactivate the remaining Brightase-C and to disperse the cell aggregate into a single cell.

Application

- When the organoids in collagen gel are recovered without disruption, they should be washed thoroughly with PBS without Trypsin/EDTA treatment after Brightase-C treatment. If cells are to be dispersed and analyzed by FACS or other means, perform Trypsin/EDTA treatment.
- The amount and digestion time of Brightase-C should be adjusted according to the type, concentration, and amount of collagen used in the gel culture.

Related product

Product code	Product name
892 101	Type I collagen, Bovine skin, Acid soluble, 3mg/mL
892 102	
892 103	Type I collagen, Bovine skin, Pepsin-solubilized, 3mg/mL
892 104	
892 107	TypeⅢcollagen, Bovine skin, Pepsin-solubilized, 3mg/mL
892 108	
892 151	TypeⅤcollagen, Bovine cornea, Pepsin-solubilized, 3mg/mL
892 111	Type I collagen, Porcine skin, Pepsin-solubilized, 3mg/mL
892 112	
892 431	Brightase-C 40mg×1 pc.
892 432	Brightase-C 40mg×2 pcs.