User Guide

Ceglu™ multiwell plate, 6well

Introduction

Ceglu™ multiwell plates are coated with a chemically synthesized scaffold material, offering excellent adhesion, uniformity, and storage stability. They are ready to use immediately after opening. This user guide provides instructions for culturing iPSCs (induced Pluripotent Stem Cells) and MSCs (Mesenchymal Stem Cells). Please note that the procedures outlined are general guidelines and may require optimization based on the specific cell line, media, or other experimental conditions. Before starting your experiment, it is recommended to perform the acclimation procedure detailed in Section 3.

Contents

Ceglu™

1.Passaging iPSCs

Plates:	Ceglu™ multiwell plate, 6well	[This product]
Cells:	Approx. 60-80% confluent human iPSCs	
Reagents:	StemFit® AK02N	[Ajinomoto Healthy Supply]
	0.5 M/L EDTA/PBS	[Nacalai Tesque]
	TrypLE™ Select (1x)	[ThermoFisher Scientific]
	CultureSure® Y-27632	[FUJIFILM Wako Chemical]
	1x D-PBS (-)	[Nacalai Tesque]

i. Single cell culture

1. Remove the medium.
2. Wash the cells with 1mL PBS and remove.
3. Add 1 mL 0.5 mM EDTA/PBS or TrypLE™ Select (1x) to each well and incubate in the 37 °C/5 % CO_2 incubator.
   Note: Optimal incubation time should be determined because dissociation time may vary depending on the cell line used.
4. After incubation, check each cells have rounded and individual cell borders are visible.
   Note: If incubation is not sufficient, incubate the plate again for 1 to 2 min.
5. Remove Dissociation solution.
6. Add 1 mL medium containing 10 µM Y-27632 and gently detach cells by pipetting.
7. Transfer the detached cells to conical tube.
8. Rinse the well with additional 1 mL medium containing Y-27632 to collect remaining cells.
9. Centrifuge the conical tube at 260 x g for 3 min at room temperature..
10. Remove supernatant not to disturb cell pellet.
11. Add 2 mL medium containing Y-27632 and suspend the cells.
12. Count the cells.
13. Seed 2 mL cell mixture at 3 x 10^4 - 1 x 10^5 cells in each well.
14. Place the plate in a 37 °C/5 % CO_2 incubator. Move the plate in several quick, short, back-and-forward and side-to-side motions to evenly distribute the cells.
15. After 24 hours, perform medium changes using the medium without Y-27632
16. Perform medium change daily until cell colonies reach approximately 60 - 80% confluent.

ii. Clump culture

1. Remove the medium.
2. Wash the cells with 1mL PBS and remove PBS
3. Add 1 mL 0.5 mM EDTA/PBS to each well and incubate in the 37 °C/5 % CO_2 incubator.
   Note: Optimal incubation time should be determined because dissociation time may vary depending on the cell line used.
4. After incubation, check each cells have rounded and individual cell borders are visible.
   Note: If incubation is not sufficient, incubate the plate again for 1 - 2 min.
5. Remove EDTA/PBS solution.
6. Detach the cells by 2 mL medium using a 5 mL pipette.
7. Transfer the detached cells to conical tube.
   Note: If necessary, repeat step 6 again.
8. Collected cell aggregates should be split 1:5 to 1:20 into this product in 2 mL medium.
9. Place the plate in a 37 °C/5 % CO_2 incubator. Move the plate in several quick, short, back-and-forward and side-to-side motions to evenly distribute the cells.
   Note: Cell aggregates should be 100 to 200 µm.
10. After 24 hours, perform medium change.
11. Perform medium change daily until cell colonies reach approximately 60 - 80% confluent.

2.Thawing iPSCs

Plates:	Ceglu™ multiwell plate, 6well	[This product]
Cells:	Cryopreserved iPSCs	
Reagents:	StemFit® AK02N	[Ajinomoto Healthy Supply]
	CultureSure® Y-27632	[FUJIFILM Wako Chemical]

1. Prepare medium 10 times of frozen stock solution containing Y-27632 in conical tube.
2. Quickly thaw cryopreserved cells in a 37 °C water bath until a small frozen portion remains.
3. Wipe outside of tubes with 70 % ethanol and transfer cells into conical tube prepared in Step 1.
4. Centrifuge the conical tube at 260 x g for 3 min at room temperature.
5. Remove supernatant not to disturb cell pellet.
6. Add 2 mL medium containing Y-27632 and suspend the cells.
7. Count the cells.
8. Seed 2 mL cell mixture at 5 x 10^4 - 1 x 10^5 cells in each well.
9. Place the plate in a 37 °C/5 % CO_2 incubator. Move the plate in several quick, short, back-and-forward and side-to-side motions to evenly distribute the cells.
10. After 24 hours, perform medium changes using the medium without Y-27632
11. Perform medium change daily until cell colonies reach approximately 60 - 80% confluent.
    Note: If you thaw the stock of clump-cell culture, use medium without Y-27632.

3. Adapting iPSCs to Ceglu™ multiwell plate

Perform 3 - 5 passages of culture using the product in the single cell or clamp culture method described above. Differentiated cells may appear in the early stages of culture, but by removing them and performing passages, the appearance of differentiated cells will decrease and proliferation will stabilize.

Reference

Shimizu, E., Iguchi, H., Le, M.N.T. et al. A chemically-defined plastic scaffold for the xeno-free production of human pluripotent stem cells. Scientific Reports 12, 2516 （2022）.

4.Passaging MSCs

Plates：	Ceglu™ multiwell plate, 6well	[This product]
Cells：	Approx. 70-80% confluent human MSCs	
Reagents：	R:STEM Medium for hMSC High Growth	[ROHTO Pharmaceutical]
	TrypLE™ Express（1x）	[ThermoFisher Scientific]
	1 x D-PBS（-）	[Nacalai Tesque]

1. Remove the medium.
2. Wash the cells with 1mL PBS and remove PBS
3. Add 1 mL TrypLE™ Express (1x) to each well and incubate in the 37 °C/5 % CO_2 incubator.
   Note: Optimal incubation time should be determined.
4. After incubation, detach the cells by gentle pipetting.
   Note: If incubation is not sufficient, incubate the plate again for 1 to 2 min.
5. Transfer cells into conical tube.
6. Rinse the well with 2 mL medium and collect cells into conical tube.
7. Transfer the detached cells to conical tube.
8. Centrifuge the conical tube at 400 × g for 5 min at room temperature.
9. Remove supernatant not to disturb cell pellet.
10. Add 2 mL medium and suspend the cells.
11. Count the cells.
12. Seed 2 mL cell mixture at 3 x 10^4 - 1 x 10^5 cells in each well.
13. Place the plate in a 37 °C/5 % CO_2 incubator. Move the plate in several quick, short, back-and-forward and side-to-side motions to evenly distribute the cells.
14. Grow cells until approximately 60 – 80 % confluent.

5. Thawing MSCs

Plates：	Ceglu™ multiwell plate, 6well	[This product]
Cells：	Cryopreserved MSCs	
Reagents：	R:STEM Medium for hMSC High Growth	[ROHTO Pharmaceutical]

1. Prepare medium ten times of frozen stock solution in conical tube.
2. Quickly thaw cryopreserved cells in a 37 °C water bath until a small frozen portion remains.
3. Wipe outside of tubes with 70 % ethanol and transfer cells into conical tube prepared in Step 1.
4. Centrifuge the conical tube at 400 × g for 5 min at room temperature.
5. Remove supernatant not to disturb cell pellet.
6. Add 2 mL medium and suspend the cells.
7. Count the cells.
8. Seed 2 mL cell mixture at 5 x 10^4 - 1 x 10^5 cells in each well.
9. Place the plate in a 37 °C/5 % CO_2 incubator. Move the plate in several quick, short, back-and-force and side-to-side motions to evenly distribute the cells.
10. Grow cells until approximately 70 – 80 % confluent.

For more information about Ceglu™ and our other products, please visit our website.

SEKISUI

SEKISUI CHEMICAL CO., LTD.

2-10-4 Toranomon, Minato-ku, Tokyo, Japan
Contact : support_life@sekisui.com