



Y-27632

Cell Passaging / Freezing / Thawing Protocol



Y-27632 is a low-molecular-weight compound that has been reported to suppress the death of human ES/iPS cells during cell dispersion and improve cell viability after cryopreservation/ thawing. Here we present a protocol for passaging, freezing, and thawing human ES/iPS cells (single cells), using our product Y-27632. See the back cover for other reagents, etc. used in the protocol.

Y-27632 products list

| Wako Cat. No. | Product Name | Grade | Package Size |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|-----------------|
| 252-00701 | Y-27632 (GMP-compliant) | | 5 mg |
| 258-00703 | This product is complied with ICH-Q7 (GMP for APIs) and is intended as a raw material for the commercial production of regenerative medicine products. | For Cell Culture | 25 mg |
| 259-00613 | Y-27632, MF This is an ISO9001-compliant product developed for the commercial manufacturing of regenerative medicine products. | | 5 mg |
| 257-00614 | | | 25 mg |
| 030-24021 | - CultureSure® Y-27632 | | 1 mg |
| 036-24023 | | | 5 mg |
| 034-24024 | | | 25 mg |
| 030-24026 | | | 100 mg |
| 039-24591 | CultureSure® 10mmol/L Y-27632 Solution, Animal-derived-free | | 300 µL |
| 035-24593 | | | 1 mL |

Preparation

1. Preparation of 10 mmol/L Y-27632 solution

This procedure is not necessary when a prepared solution product (039-24591, 035-24593) is used.



Dissolve Y-27632 (powder) in sterile water to a concentration of 10 mmol/L (3.38 g/L).



Filter the solution through a 0.22 μm filter.

The procedures should be performed in a safety cabinet, on a clean bench, etc.

When stored, the solution should be dispensed into single-use portions in sterilized containers and cryopreserved.

2. Addition of Y-27632 solution to the medium



 Add 10 mmol/L Y-27632 solution to give a final volume of 1/1,000 that of the medium (final concentration: 10 μmol/L).

Mix well.

Human ES/iPS cell culture medium (hPSC medium)

Hereinafter, the hPSC medium supplemented with Y-27632 is referred to as "hPSC medium+Y".

Cell Passaging (single cells)

For one 10-cm dish



Remove the medium from the culture dish.





Add 5 to 10 mL of D-PBS (-) to the dish and rinse.



Remove D-PBS (-).



Add 2 to 5 mL of a cell dispersing agent.



Allow to stand in an incubator set to 5% CO₂, 37 °C.



Add 10 mL of hPSC medium+Y to the dish.



Disperse the human ES/iPS cell colonies into single cells by pipetting.



Transfer the medium with dispersed cells into a tube.



Centrifuge the tube for 5 min at 200 x g, room temperature, and remove the supernatant.



Add 10 mL of hPSC medium+Y to the tube to suspend the cell pellet.



Count cells.



Seed an appropriate amount of human
 ES/iPS cells in a new culture dish previously containing hPSC medium+Y.



Culture in an incubator set to 5% CO₂, 37 °C.



The following day, replace the medium with hPSC medium without Y-27632.

Cell Freezing (single cells)

For one 10-cm dish



Remove the medium from the culture dish.





Add 5 to 10 mL of D-PBS (-) to the dish and rinse.



Remove D-PBS (-).



Add 2 to 5 mL of a cell dispersing agent.



Allow to stand in an incubator set to 5% CO₂, 37 °C.



Add 10 mL of hPSC medium+Y to the dish.



Disperse the human ES/iPS cell colonies into single cells by pipetting.



Transfer the medium with dispersed cells into a tube.



Centrifuge the tube for 5 min at 200 x g, room temperature, and remove the supernatant.



Add 10 mL of hPSC medium+Y to the tube to suspend the cell pellet.



Count cells.



Centrifuge the tube for 5 min at 200 x g, room temperature, and remove the supernatant.



Add an appropriate amount* of cell preservation solution to suspend the cell pellet.

*e.g., 1 x 106 cells/mL



Portion the cell suspension in appropriate* volumes in cryopreservation vials.

*e.g., 500 μL/vial



Cell Thawing



Pre-warm hPSC medium+Y to 37°C.



Frozen cells

Add the hPSC medium+Y to thaw the frozen cells.

> Transfer the cells into a centrifuge tube containing 10 mL of hPSC medium+Y.

Centrifuge the tube for 5 min at 200 x g, room temperature, and remove the supernatant.



Add 10 mL of hPSC medium+Y to the tube to suspend the cell pellet.



Count cells.



Seed an appropriate amount of human ES/iPS cells in a new culture dish previously containing hPSC medium+Y.



Culture in an incubator set to 5% CO₂, 37°C.



The following day, replace the medium with hPSC medium without Y-27632.

Related Products

| Wako Cat. No. | Product Name | Grade | Package Size |
|---------------|--------------------------------------------------------------------------------------------------------------------|------------------|-----------------|
| 033-26071 | CultureSure™ CEPT Cocktail (1,000×) | For Cell Culture | 300 µL |
| 197-17571 | StemSure [®] hPSC Medium Δ | For Cell Culture | 100 mL |
| 193-17573 | This product does not contain bFGF (basic fibroblast growth factor). | For Cell Culture | 100 mL x 4 |
| 064-05381 | Fibroblast Growth Factor (basic)(FGF-basic / bFGF / FGF2), Human, recombinant, Animal-derived-free(154aa) (powder) | For Cell biology | 50 µg |
| 068-05384 | | | 100 µg |
| 060-05383 | | | 1 mg |
| 039-24155 | CultureSure® Sterile Water (Endotoxin and Mycoplasma Free) | For Cell Culture | 500 mL |
| 045-29795 | D-PBS(-) | | 500 mL |
| 043-29791 | | | 1 L |
| 049-29793 | | | 500 mL x 10 |
| 203-20251 | T : FDTA 0 (| | 100 mL |
| 205-20255 | Trypsin-EDTA Solution without Phenol Red, AF | | 500 mL |
| 220-02041 | Vitronectin(20-398 aa), Human, recombinant, Solution Dish coating material | For Biochemistry | 500 µg |

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit FUJIFILM Wako Laboratory Chemicals site: https://labchem-wako.fujifilm.com/ / This leaflet may contain products that cannot be exported to your country due to regulations. / Bulk quote requests for some products are welcomed. Please contact us.

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