



# 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<br>Size |
|---------------|---|---------------------|-----------------|
| 252-00701     | Y-27632 This product complies with ICH-Q7 (GMP for API) guidelines as a raw material for commercial production of regenerative medicine products. | For<br>Cell Culture | 5 mg            |
| 258-00703     |   |                     | 25 mg           |
| 259-00613     | Y-27632, MF This product complies with ISO9001. The manufacturing process and analytical procedure are validated.                                 |                     | 5 mg            |
| 257-00614     |   |                     | 25 mg           |
| 039-24591     | CultureSure® 10mmol/L Y-27632 Solution, Animal-derived-free   |                     | 300 µL          |
| 035-24593     |   |                     | 1 mL            |
| 030-24021     | - CultureSure® Y-27632  |                     | 1 mg            |
| 036-24023     |   |                     | 5 mg            |
| 034-24024     |   |                     | 25 mg           |
| 030-24026     |   |                     | 100 mg          |

### **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  $\mu m$  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% CO<sub>2</sub>, 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% CO<sub>2</sub>, 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% CO<sub>2</sub>, 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% CO<sub>2</sub>, 37°C.



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

#### **Related Products**

| Wako Cat. No. | Product Name   | Grade            | Package<br>Size |
|---------------|--|------------------|-----------------|
| 033-26071     | CultureSure™ CEPT Cocktail (1,000×)  | For Cell Culture | 300 μL          |
| 197-17571     | StemSure® hPSC Medium $\Delta$ This product does not contain bFGF (basic fibroblast growth factor).                | For Cell Culture | 100 mL          |
| 193-17573     |  |                  | 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<br>(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     | Townsin EDTA Calation with ant Dhamal Dad AF   |                  | 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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