



Y-27632

Cell Passaging / Freezing / Thawing Protocol



Y-27632 is a small molecular 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 is a protocol for passaging, freezing and thawing human ES/iPS cells for single cells and using our Y-27632.

Y-27632 products list

Wako Cat. No.	Product Name	Grade	Package Size
030-24021	- CultureSure® Y-27632 (powder)	For Cell Culture	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
259-00613	Y-27632, MF (powder)		5 mg
257-00614			25 mg

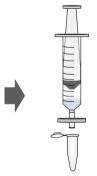
Preparation

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 μ m filter.

The procedures should be performed in a biological 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



Human ES/iPS cell culture medium (hPSC medium) Add 10 mmol/L Y-27632 solution to the medium at final concentration, Medium:Y-27632 = 1,000:1. (* final concentration: 10 μ mol/L).

Pipetting well.

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 and suspend the cell pellet.



Count cells.



Seed an appropriate amount of human ES/iPS cells in a new culture dish pre-dispensed 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 and 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

Store at the recommended temperature for cryopreservation. For long-term storage, store in -150°C or liquid nitrogen.

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 pre-dispensed 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
197-17571	StemSure [®] hPSC Medium Δ 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 Cellbiology	50 µg
068-05384			100 µg
060-05383			1 mg
039-24155	CultureSure [®] Sterile Water (Endotoxin and Mycoplasma Free)		500 mL
045-29795		For Cell Culture	500 mL
043-29791	D-PBS(-)		1 L
049-29793			500 mL x 10
203-20251	Trypsin-EDTA Solution without Phenol Red, AF		100 mL
205-20255			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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