



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)	For Cell Culture	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.		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

Store at the recommended temperature for cryopreservation. For long-term strage, 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 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	Trypsin-EDTA Solution without Phenol Red, AF		100 mL
205-20255			500 mL
195-16031	StemSure® Freezing Medium		100 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.

FUJIFILM Wako Laboratory Chemicals site https://labchem-wako.fujifilm.com

1-2, Doshomachi 3-Chome, Chuo-ku, Osaka 540-8605, Japan Tel: +81 6 6203 3741 Fax: +81 6 6203 1999 ffwk-cservise@fujifilm.com

FUJIFILM Wako Chemicals U.S.A. Corporation ToJIFILM WAKO CHEMICAIS U.S.A. 1600 Bellwood Road, Richmond, VA 23237, U.S.A. 1610-Free (U.S. only): +1 877 714 1920
Tel: +1 804 271 7677 Fax: +1 804 271 7791
wkuslabchem@fujifilm.com

FUJIFILM Wako Chemicals (Hong Kong) Limited

Room 1111, 11/F, International Trade Centre, 11-19 Sha Tsui Road, Tsuen Wan, N.T., Hong Kong Tel: +852-2799-9019 Fax: +852-2799-9808 wkhk.info@fujifilm.com

FUJIFILM Wako Chemicals Europe GmbH

Fuggerstr 12, 41468 Neuss, Germany Tel: +49 2131 311 0 Fax: +49 2131 311 100 labchem_wkeu@fujifilm.com

Tel: +86-10-6413-6388(Beijing) wkgz.info@fujifilm.com

FUJIFILM Wako (Guangzhou) Trading Corporation

Room 3003, 30/F., Dong Shan Plaza 69, Xian Lie Zhong Road Guangzhou, 510095, China Tel: +86-20-8732-6381(Guangzhou) Tel: +86-21-6288-4751(Shanghai)

23700学21