



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 This product complies with ICH-Q7 (GMP for API) guidelines as a raw material for commercial production of regenerative medicine products.	For 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 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".





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.

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.



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.





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.



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

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.



Related Products

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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 Coll Culture	100 mL
193-17573	This product does not contain bFGF (basic fibroblast growth factor).		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

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