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STEMUP™

ES/iPS cell culture medium supplement

Protocol

FOR RESEARCH USE ONLY

Nissan Chemical
CORPORATION

Table of contents

1. Introduction

STEMUP is a cell culture medium to culture ES/iPS cells. It is a xeno and albumin free medium that is chemically defined and designed to enhance homogeneity among ES/iPS cell population. It can be easily mixed with DMEM/F-12 basal media to yield a 2D cell culture medium.

Applications & Typical Use:

- To culture undifferentiated ES/iPS cells for mass production
- To effectively maintain ES/iPS cells under feeder-free condition

Benefits:

- Chemically defined medium with low concentration of growth factors
- Xeno free & Albumin free
- Supports cell growth performance
- Applicable to various kind of ES/iPS cell lines
- Applicable to 3D cell culture using FCeM® FP polymer
- Cost effective against competitive products
- Preparing clinical grade

8mL solution for 500mL medium

【Contents】

	Contents	Appearance	Preservation	Shelf life
STEMUP™ supplement	8 mL	Liquid, colorless	freeze (-20℃)	For 12 months

【Cautions】

- This product (“Product”) is designed for research and development use only – Do not use it for other purposes.
- Wear appropriate protective eyewear, clothing, and gloves when handling the Product.
- Avoid skin and eye contact, inhalation of vapors, or ingestion.
- No warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non-infringement, except as provided for herein.
- Nissan Chemical Corporation shall not be liable for any damages as the result of (i) misuse, fault or negligence of or by users or purchasers of the Product, (ii) use of the Product in a manner for which it was not designed, or (iii) improper storage and handling of the Product.

2. Materials and equipment

- 8mL STEMUP™ supplement (for 500mL basal medium)
- DMEM/F-12, HEPES (ThermoFisher, #11330032, 500mL)
- Vitronectin (VTN-N) (ThermoFisher, #A14700, ×100, final conc. 5μg/mL)
- Y-27632 (Wako, #034-24024, dilution with D-PBS(-) to 10mM(×1000), final conc. 3-10μM)
- D-PBS(-)
- Dissociation reagent; 0.5mM EDTA/PBS solution (Versene Solution, ThermoFisher, #15040066)
- STEM-CELLBANKERR GMP grade (Nippon Zenyaku Kogyo, #CB045)
- 6-well plate
- 15mL and 50mL tubes
- Hemocytometer or Cell counter
- Other required culture equipment

3. Culture schedule example (in case of using 6-well plate)

	Mon	Tue	Wen	Thu	Fri	Sat	Sun
Operation	**Passage**	Medium change	Medium change	**Passage**	Medium change	Medium change	Medium change
Seeding density※2	1-3× 10^5 cells/well	-	-	0.5-2× 10^5 cells/well	-	-	-
ROCK inhibitor (Y-27632)	3-10μM※3	-	-	3-10μM※3	-		
Operation	**Passage**	Medium change	-	**Passage**	Medium change※1	Medium change※1 (Either one day)	
Seeding density※2	1-3× 10^5 cells/well	-	-	0.5-2× 10^5 cells/well	-	-	
ROCK inhibitor (Y-27632)	3-10μM※3	-	-	3-10μM※3	-	-	

※1 By doubling the amount of medium on previous day, skip of medium change is possible for a day
e.g. Skip of Saturday's operation…Medium change 4mL/well on Friday

※2 Seeding density of thawed frozen cells : 3-5×10^5cells/well

※3 The optimal ROCK inhibitor (Y-27632) concentration depends on the cells and culture conditions.

4. Preparation of STEMUP™ medium

Materials

- DMEM/F-12, HEPES (ThermoFisher, #11330032, 500mL)
- 8mL STEMUP™ supplement (for 500mL basal medium)

Operations

1. Thaw STEMUP™ supplement slowly at 2ºC to room temperature.
2. Add STEMUP™ supplement to DMEM/F-12.
3. Rinse the supplement bottle with STEMUP™ medium and add to the STEMUP™ medium.
4. Gently swirl complete STEMUP™ medium to mix and store at 4ºC until using.
5. STEMUP™ medium is stable for up to 1 months when stored at 4ºC.
6. Divide required medium into usage-size tube, and warm it at room temperature.
   ※Do not warm the medium at 37ºC.

5. Coating culture vessels with vitronectin (VTN-N)

Materials

- Vitronectin (VTN-N) (ThermoFisher, #A14700, ×100, final conc. 5μg/mL)
- D-PBS(-)
- 6-well plate

Operations

1. Add 1.5mL D-PBS(-) with 15μL Vitronectin (VTN-N) to one well of a 6-well plate
2. Incubate at room temperature for 60 minutes

6. Thawing cells

Materials

- STEMUP™ medium
- Y-27632 (Wako, #034-24024, dilution with D-PBS(-) to 10mM(×1000), final conc. 3-10μM)
- Coating 6-well plate with Vitronectin (VTN-N)

Operations

1. Warm required STEMUP™ medium at room temperature.
2. Add 2mL STEMUP™ medium (including 3-10μM Y-27632) to a well of coating 6-well plate with Vitronectin (VTN-N)
3. Divide about 10mL STEMUP™ medium to tube, and pre-warm it.
4. Immerse the frozen vial in a 37ºC water bath, and remove the vial from the water bath before thawing the cells completely.
5. Transfer the thawed cells to a 15mL tube and slowly add 10mL pre-warm STEMUP™ medium to the cells.
6. Centrifuge the cells at 200×*g* for 2 minutes.
7. Aspirate and discard the supernatant, and resuspend the cell pellet in STEMUP™ medium (including 3-10μM Y-27632) by gently pipetting, then count the cells.
8. Seed the cell suspension into VTN-N-coated 6-well plate with STEMUP™ medium (including 3-10μM Y-27632), plating 3-5×10^5cells per well.
9. Place the plate in a 37ºC incubator, with humidified atmosphere of 5% CO_2.
10. The next day, replace the spent medium with fresh complete STEMUP™ medium (not including Y-27632). Replace the medium dairy thereafter.

7. Medium change

Materials

- STEMUP™ Medium

Operations

1. Warm required medium at room temperature.
2. Aspirate and discard the spent medium, and add the fresh medium (not including Y-27632) to the well.

8. Passaging

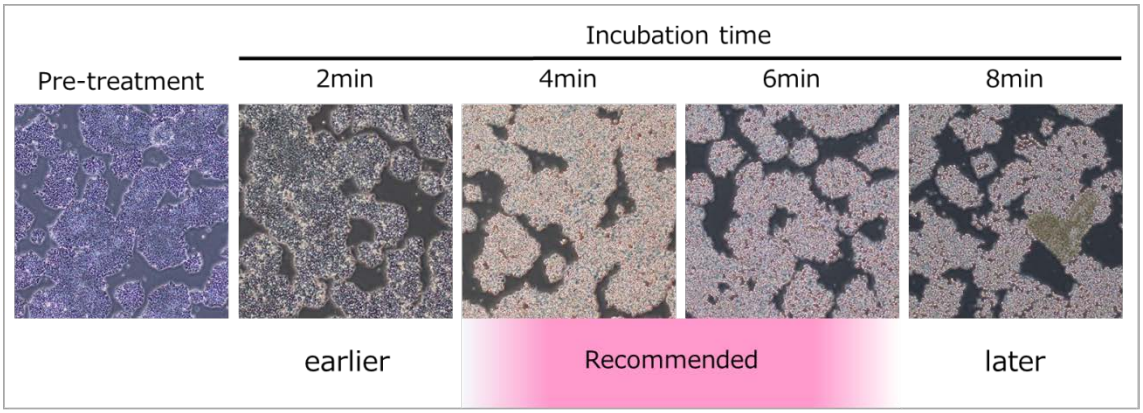
Materials

- D-PBS(-)
- STEMUP™ medium
- Y-27632 (Wako, #034-24024, dilution with D-PBS(-) to 10mM(×1000), final conc. 3-10μM)
- Dissociation reagent; 0.5mM EDTA/PBS solution (Versene Solution, ThermoFisher, #15040066)
- Coating 6-well plate with Vitronectin (VTN-N)

Operations

1. Warm required medium, D-PBS(-) and dissociation reagent at room temperature.
2. Add 2mL STEMUP™ medium (including 10μM Y-27632) to a well of coating 6-well plate with Vitronectin (VTN-N).
3. Aspirate the spent medium from each well containing the cells, and rinse each well once with D-PBS(-).
4. Add 0.5mL dissociation reagent to each well containing the cells, and incubate the plate at 37 ºC for 4 to 6 minutes. (**See below**)
5. Confirm that the cells starts to dissociate when viewed under microscope, and aspirate the dissociation reagent.
6. Add pre-warmed 1mL STEMUP™ medium to each well, and remove the cells from the well(s) by gently pipetting the colonies up. Collect the cells in a 15mL tube.
7. Repeat the operation of step-6.
8. After gently pipetting, count the cells.
9. Transfer the appropriate volume of cell suspension into VTN-N-coated 6-well plate with STEMUP™ medium (including 10μM Y-27632).
   (seeding density: 0.5-3×10^5 cells/well, refer to Section 3. ”Culture schedule example”)
10. Place the plate in a 37ºC incubator, with humidified atmosphere of 5% CO_2.
11. The next day, replace the spent medium with fresh complete STEMUP™ medium (not including Y-27632). Replace the medium dairy thereafter.

▼Effect of dissociation reagent on incubation time and morphology of the cells



- The optimal incubation time may vary when using different cell lines or culture conditions of the cells, therefore dissociation should be monitored under the microscope every time.
- In most cases, adding medium to the plate can easily detach the cells, but it may be necessary to extend the incubation time or scrape with a scraper depending on cell lines.

9. Cryopreserving cells

Materials

- D-PBS(-)
- STEMUP™ medium
- Dissociation reagent; 0.5mM EDTA/PBS solution (Versene Solution, ThermoFisher, #15040066)
- STEM-CELLBANKERR GMP grade (Nippon Zenyaku Kogyo, #CB045)

Operations

1. Warm required medium, D-PBS(-) and dissociation reagent at room temperature.
2. Aspirate the spent medium from each well containing the cells, and rinse each well once with D-PBS(-).
3. Add 0.5mL dissociation reagent to each well containing the cells, and incubate the plate at 37 ºC for 4 to 6 minutes.
4. Confirm that the cells starts to dissociate when viewed under microscope, and aspirate the dissociation reagent.
5. Add pre-warmed 1mL STEMUP™ medium to each well, and remove the cells from the well(s) by gently pipetting the colonies up. Collect the cells in a 15mL tube.
6. Repeat the operation of step-5.
7. After gently pipetting, count the cells.
8. Centrifuge the cells at 200×*g* for 2 minutes.
9. Aspirate the medium, and resuspend the cell pellet in cold STEM-CELLBANKER® by gently pipetting. The cell Density should be less than 2.0×10^6 cells/mL.
10. Transfer the appropriate volume of stock solution into cryovial.
11. Freeze the stock solution at -80°C, and transfer into liquid nitrogen after the next day.

*If you have any questions related to these instructions, encounter problems,
or need help, please contact us by e-mail, phone, or fax.

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