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# WTc11 iPSC Culture and Maintenance


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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol explains general culture and maintenance of the WTc11 iPSC cell line.

## Attachments

DOCX

WTc11 iPSC CULTURE S...

17KB

## Materials

MATERIALS

- StemPro™ Accutase™ Cell Dissociation Reagent **Thermo Fisher Scientific** **Catalog #**A1110501
- DPBS, no calcium, no magnesium **Thermo Fisher** **Catalog #**14190136
- Trypan Blue Solution, 0.4% **Thermo Fisher** **Catalog #**15250061
- StemFlex™ Medium **Thermo Fisher Scientific** **Catalog #**A3349401
- Matrigel **Corning** **Catalog #**356231
- KnockOut™ DMEM **Thermo Fisher Scientific** **Catalog #**10829018
- Rock Inhibitor Y-27632 Dihydrochloride **Tocris** **Catalog #**1254

## Safety warnings

Please refer to the Safety Data Sheets (SDS) for safety and health data.

## Before start

Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch.

Alternatively, an aliquot for use that day may be pre-warmed in a 37 °C waterbath until no longer cool to the touch. Avoid extended dwell times at 37 °C.

## Prepare complete StemFlex Medium

1 Thaw the frozen StemFlex Supplement 10X at Room temperature for ~ 02:00:00 or overnight at 2 °C to 8 °C .

Note

IMPORTANT! Do not thaw the frozen supplement at 37°C.

2 Mix the thawed supplement by gently inverting 3−5 times.

3 Aseptically transfer 50 mL of StemFlex Supplement 10X to the bottle of StemFlexTM Basal Medium (450 mL fill).

4 Gently invert the bottle several times to obtain 500 mL of homogenous complete medium.

Note

Following reconstitution, complete StemFlexTM Medium can be stored at 2°C to 8°C for up to 2 weeks or aliquoted and stored at -5 °C to -20 °C for up to 6 months. Alternatively, usage size aliquots of the supplement can be made and frozen at -5 °C to -20 °C for up to 6 months. Avoid multiple freeze-thaw cycles.

## Feeding WTc11 iPSC

5 Feed the PSCs the day after seeding followed by every-other-day thereafter.

Note

If the cells are to be left without feeding for two days (for example, over a weekend), then double the feed volume (i.e., 4 mL added per well of 6-well plate).

## Passaging WTc11 iPSC

6 iPSCs should be split when cells are ~ 80% confluent.

7 Thaw Matrigel on ice and dilute in pre-chilled Knockout DMEM for a final volume of [M] 100 microgram per milliliter (µg/mL) .

8 Coat desired wells/plates with diluted Matrigel and incubate at 37 °C for 00:30:00 - 01:00:00 using the following table for volumes to add **per well**:

| | Per: | 96-well | 24-well | 12-well | 6-well | 10-cm dish | 15-cm dish |
|---|---|---|---|---|---|---|---|
| | **Volume to add:** | 40 µL | 200 µL | 0.5 mL | 1 mL | 5 mL | 10 mL |

Note

Matrigel may be re-used during this time only to coat additional plates. Original plates should
have PBS or media to prevent the matrix from drying out. Matrigel coated plates must be used within 14 days of coating.

9 Tilt cell-containing plate towards you and aspirate existing media.

10 Wash wells once with ample PBS (about 2x amount of media).

11 Add accutase to well(s) using the following table for volumes per well and incubate at 37 °C for 00:03:00 ; add another 01:00:00 - 00:02:00 if cells have not mostly lifted/dissociated.

| | Per: | 96-well | 24-well | 12-well | 6-well | 10-cm dish | 15-cm dish |
|---|---|---|---|---|---|---|---|
| | **Volume to add:** | 20 µL | 100 µL | 250 µL | 0.5 mL | 2 mL | 4 mL |

12 Add ample PBS to accutase-containing well(s) to dilute accutase using the following table for volumes per well:

| | Per: | 96-well | 24-well | 12-well | 6-well | 10-cm dish | 15-cm dish |
|---|---|---|---|---|---|---|---|
| | **Volume to add:** | 200 µL | 1 mL | 2.5 mL | 5 mL | 10 mL | 20 mL |

13 Pipette up and down gently to mechanically release remaining cells, collect, and add to appropriately-sized conical tubes.

14 Spin cells at 200 x g for 00:05:00 at Room temperature .

15 Carefully aspirate supernatant from pelleted conicals.

16 Add appropriate volume of StemFlex + Rock inhibitor at [M] 10 micromolar (µM) to conicals according to pellet size for counting.

Note

Rock inhibitor should only be used when cells are individualized or in small colonies (typically the first two days after passaging); the presence of Ri at higher densities results in cell stress/death, and in general, Rock inhibitor greatly reduces proliferation.

Note

For first time use of Rock inhibitor, it is suggested to aliquot at 10mM [1000x], diluting in DPBS, and use on cells at concentration [M] 10 Mass Percent .

17 Triturate to resuspend cells in StemFlex + Rock inhibitor and remove 10µL and add this volume to a 1.5mL Eppendorf tube.

Note

Be careful to minimize contact of pipette with the side of the conical wall.

18 Count cells and calculate desired number of cells to seed, and dilute this volume with additional StemFlex + Rock inhibitor to plate using the following table for volume to add per well:

| | Per: | 96-well | 24-well | 12-well | 6-well | 10-cm dish | 15-cm dish |
|---|---|---|---|---|---|---|---|
| | **Volume to add:** | 50-100 µL | 250-500 µL | 0.75-1 mL | 1.5-2 mL | 8-12 mL | 15-25 mL |

Note

For general passaging where exact cell number seeded is not important, adding resuspended cells at 1:100, 1:50, and 1:20 the final well volume typically provides near-confluency in 7 days, 5 days, and 3 days, respectively.

19 iPSCs can be frozen in StemFlex + 10% DMSO.