



Oct 25, 2019

WTc11 iPSC Culture and Maintenance

Connor Ludwig¹, Ruilin Tian¹, Martin Kampmann¹

¹University of California, San Francisco

1 Works for me dx.doi.org/10.17504/protocols.io.8rhv36

Neurodegeneration Method Development Community KampmannLab



Martin Kampmann
University of California, San Francisco

ABSTRACT

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Tian et al (2019). CRISPR Interference-Based Platform for Multimodal Genetic Screens in Human iPSC-Derived Neurons. Neuron pii: S0896-6273(19)30640-3. [Epub ahead of print] PubMed PMID: 31422865.

WTc11 iPSC CULTURE
SOP_RT_updated.docx

MATERIALS

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

SAFETY WARNINGS

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

BEFORE STARTING

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**.



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 StemFlex™ Basal Medium (450 mL fill).

- 4

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



Following reconstitution, complete StemFlex™ 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 iPSCs the day after seeding followed by every-other-day thereafter.



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 **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



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 **10 Micromolar (μM)** to conicals according to pellet size for counting.

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.



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

17 

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




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



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.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited