[Protocol 1.03.0] Passaging of iPSC into single cells using TrypLE/Accutase

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Version: 1.0 (20.12.2021)

Media and Reagents:
TrypLE-Select (1x) (Thermo Fisher, 12563011)
Accutase (Stem Cell Technologies, 07920 or Thermo Fisher A1110501)
PBS w/o Ca2+Mg2+ (Thermo Fisher, 14190250)

Medium (Thermo Fisher, A15171-01)
prepare maintenance medium in regarding protocol 1.07, 1.08 or 1.09
Y-27632 ROCK inhibitor (Stem Cell Technologies,72305 or Selleck Chemicals, SEL-S1049-10mM)
add ROCK inhibitor regarding protocol 1.03.1

Materials and Equipment:
Geltrex coated culture vessels(*refer to protocol 1.05*)
Aspiration Pump
5ml/10ml pipettes
Cell counting equipment
15/50 ml Falcon tube

1. Introduction and Purpose
This protocol describes the passaging procedure of induced pluripotent stem cells (iPSC) into single cells using Accutase or TrypLE-Select. The passaging with TrypLE /Accutase results in having majorly single cells in the culture, instead of cell clumps (= aggregation of single cells). This is the main feature of passaging with TrypLE /Accutase compared to the passing with EDTA, and this is because we aim to have an accurate cell number for certain experiments, i.e. to control the number of seeded cells. Unlike the passaging with TrypLE/Accutase, the EDTA passaging intends to produce more cell clumps to increase the cell viability for the maintenance (*refer to protocol 1.02.0*).

2. Procedure
1. Pre-warm an appropriate volume of the medium with ROCK inhibitor to room temperature (22°± 2°C) by placing outside the fridge for at least 20-30min. (not more than 3 hours).
2. If cells will be passaged to a 6 well plate or a vessels with larger surface, prepare the previously coated culture vessels by aspirating residual coating solution and adding 3⁄4 of the final media volume (*see Table below*).
3. Place the vessel with media into the incubator (pre-warming to 37°C) until cell seeding.
4. Aspirate medium from the culture and rinse once with appropriate volume PBS (*see table below*).
5. Add Accutase or TrypLE-Select to the wells (see table below for suggested volumes).

Culture Vessel	Surface Area [cm2]	PBS [ml]	Accutase/TrypLE [ml]	Media + ROCK(re-suspending) [ml]	Mediumvolume pre-fill/ well [ml]	Final volume/ well [ml]
96 well	0,3	0,15	0,05	0,15		0,2
24 well	2	0,5	0,25	1		0,5
12 well	4	1	0,5	1,5		1
6 well	10	2	1	3	1-1,5	2
T25	25	6	2	6	3,3	5

6. Place cells at 37°C for 2-6 minutes. Check under the microscope every 2 min if cells are rounded and dissociated. If necessary tap culture vessel to detach all cells.
7. Add the appropriate amount of the medium (see table above) supplemented with ROCK inhibitor (10 µM). Detach and dissociate cells by pipetting up and down several times.
Note: If necessary re-suspend cells by using a 1000 µl pipette and gently pipetting up and down several times to get single cells. Continue immediately with next step **critical for cell viability!**
8. Transfer cell suspension to a 15 or 50 ml falcon tube and centrifuge at 300g for 3-5minutes.
9. Aspirate supernatant and re-suspend cell pellet in an appropriate volume (see table above) of pre-warmed culture medium supplemented with ROCK inhibitor (10 µM).
10. Perform a cell count.
11. Calculate the volume of cell suspension containing the number of cells needed. Seeding density should be 1.0 – 2.0 x 10^4 cells per cm2 (e.g. in a 6 well plate seed 1.0 – 2.0 x10^5 cells/well).
12. Pipette cell suspension into the new Geltrex coated (pre-filled) wells. Rock the plate several times from side to side, back and forth to distribute the cells evenly over the culture surface.
Note: For smaller vessel types, it is also possible to make up the volume of the cell suspension using pre-warmed culture medium supplemented with ROCK inhibitor to the required amount needed for seeding into new culture dishes.

13. Place the culture dish in the incubator either Normixia or Hypoxia.
14. After 24 h, change media without ROCK inhibitor.

Note: Cells show a different morphology when cultured in medium containing ROCK inhibitor. This morphology will disappear after changing back to medium without ROCK inhibitor.

Relevant applicable documents:

Protocol 1.03.1 Reconstitution, aliquoting and use of Y26732 ROCK inhibitor
Protocol 1.05 Geltrex coating of culture vessels
Protocol 1.07 Preparation of E8 medium
Protocol 1.08 Preparation of mTeSR1 medium
Protocol 1.09 Preparation of stemMACS/UPPS medium