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Single cell passaging of hPSC

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Valeria Fernandez Vallone[1], Lyn Healy[2], Nathalie Lefort[3], Katarzyna Ludwik[1], Tamer Onder[4], Lisa Pavinato[5,6], Fatma Visal FVO OKUR[7], Harald Stachelscheid[1]

[1]Core Unit pluripotent Stem Cells and Organoids - Berlin Institute of Health @ Charite, Berlin, Germany;
[2]The Francis Crick Institute;
[3]Université de Paris, Imagine Institute, iPSC Core Facility, INSERM UMR U1163, F-75015 Paris, France.;
[4]Koc University;
[5]Institute of Oncology Research (IOR), Bellinzona Institutes of Science (BIOS+), Bellinzona, Switzerland;
[6]Faculty of Biomedical Sciences, Università della Svizzera Italiana, Lugano, Switzerland.;
[7]Hacettepe University, Center for Stem Cell Research and Development (PEDISTEM) and Hacettepe University Faculty of Medicine, Department of Pediatrics

Harald Stachelscheid
Core Unit pluripotent Stem Cells and Organoids - Berlin Inst...

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We use this protocol and it's working

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Abstract

This protocol describes single cell passaging of hPSC using enzymatic dissociation reagents.

Guidelines

The protocol is adaptable to different enzyme-free reagents described in Materials section and various vessel formats.

Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 1/5/10 mL pipettes
- 15/50 mL conical tubes
- Cell culture treated plastic vessels of choice e.g. 24, 12 or 6-well plates, T25, T75 flasks, 10cm dishes
- 10/200/1000µL tips and micropipettes (optional)
- Cell scraper
- Cell counting equipment
- Aspirator pump with disposable pipette
- Centrifuge
- Microscope, if available Stereo Microscope
- Incubator at 37^{o}C and 5% CO_2 or under hypoxic conditions, 5% O_2/ 5% CO_2
- Class II Biosafety Cabinet

MEDIA AND REAGENTS

StemPro™ Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher Catalog #**A1110501

TrypLE™ Express Enzyme **Thermo Fisher Scientific Catalog #**12604013

Gentle Cell Dissociation Reagent 100 mL **STEMCELL Technologies Inc. Catalog #**7174

Trypan Blue Solution 0.4% Sterile-filtered **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8154

hPSC culture conditions and survival factors choice depend on hPSC line and individual lab practices. For options refer to protocols:
Maintenance of hPSC
Coating of tissue Culture Vessels for hPSC maintenance
Survival factors for hPSC growth

Preparation of destination vessel

30m

1 Prepare coated tissue culture vessels and culture media according to hPSC line requirements and desired format (**Table 1**).
Refer to protocols: **Coating of tissue Culture Vessels for hPSC** and **Maintenance of hPSC**.

	A	B	E	F
	Culture vessel	Dissociation reagent (mL)	hPSC media + survival factor (harvest: mL/per well)	hPSC media + survival factor (final: mL/per well
	96 well	0.05	0.15	0.2
	24 well	0.25	1	0.5
	12 well	0.5	1.5	1
	6 well	1	3	2
	T25	2	6	7

Table 1. Recommended volumes according to vessel format

2 Equilibrate prepared destination vessel at 37 °C until usage. 30m

hPSC single-cell passaging

1h 3m

3 Prepare required volume of the reagents and media according to the **Table 1.** Equilibrate the media to Room temperature . 20m

Note

Culture media aliquot to be used can be warmed up at 37°C for 00:15:00 . However, to preserve recombinant proteins activity Room temperature equilibration is recommended.

4 Aspirate and discard medium from the selected source vessel. 1m

5 Add enzymatic dissociation reagent, refer to **Table 1** for recommended working volumes. 1m

6 Incubate the vessel at 37 °C for 00:05:00 - 00:10:00. 10m

Note

Incubation time depends on reagent used, coating and hPSC line attachment properties. Therefore, monitoring under microscope every 2-3 min until hPSC detachment observation is recommended.
If necessary, gently tap culture vessel from the side to detach hPSC.

7 Add hPSC media supplemented with survival factors to the wells containing dissociation reagent, refer to **Table 1** for recommended harvesting volumes. 1m

8 Pipette up and down several times to dislodge the cells from the culture surface. 3m

Note

Pipette selection for this step depends on total hPSC suspension volume. The smaller the tip of the pipette the better cells will be singularized.

9 Transfer hPSC suspension to a 15 or 50 mL conical tube. 2m

Note

Several wells or vessels can be combined for centrifugation step.

10 Centrifuge at 300 x g for 00:05:00. 5m

11 Aspirate and discard supernatant without disrupting the cell pellet. 1m

12 Resuspend cell pellet in hPSC media supplemented with survival factors. Resuspension volume will depend on pellet size in a range of 1-10 mL. 2m

13 Perform hPSC counting using viability staining such as Trypan Blue 0.4%. 10m

Note

Counting can be performed manually using e.g. Neubauer Chamber or automated cell counting devices.

14 Adjust hPSC suspension volume according to desired number of cells for seeding, refer to **Table 1** for recommended final media volumes. 2m

Note

As example, for hPSC single cell passaging for hPSC maintenance in 6 well-plates, we recommend a seeding density between 200,000-400,000 cells per well. Seeding densities have to be tested and adjusted per hPSC line for optimal passaging.

15 Aspirate coating solution from the destination vessel. 1m

16 Pipette cell suspension into the coated recipient wells according to desired hPSC seeding density, refer to **Table 1** for recommended final media volumes. 3m

17 Gently move destination vessels with hPSC in suspension in cross shape to distribute them evenly. 1m

18 Incubate the vessels at 37 °C and at [M] 5 % volume CO_2.

19 After 24:00:00, perform full media change with respective hPSC maintenance media without survival factors. For further hPSC culture refer to protocol **Maintenance of hPSC**.

Note

hPSC display different morphology when cultured in medium containing survival factors. This morphology will change after media is replaced with medium without survival factors.
For detailed morphology, refer to protocol **Reference pictures of hPSC cultured in defined conditions**