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Non-enzymatic passaging of hPSC

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We use this protocol and it's working

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Abstract

This protocol describes the procedure to passage hPSC culture using enzyme-free reagents.

Guidelines

In this protocol, hPSC passaging is done in aggregate/clumps format. The protocol is adaptable to different enzyme-free reagents described in Materials section and variable plate 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
- Aspirator pump with disposable pipette
- Sterile filter unit with 0.22 µm filter
- 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

EDTA (0.5 M), pH 8.0 **Life Technologies Catalog #**AM9260G

ReLeSR™ 100 mL **STEMCELL Technologies Inc. Catalog #**5872

Versene Solution **Thermo Fisher Catalog #**15040033

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

DPBS no calcium, no magnesium **Invitrogen - Thermo Fisher Catalog #**14190136

UltraPure Distilled Water **Invitrogen - Thermo Fisher Catalog #**10977-015

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

Before start

If using EDTA as enzyme-free reagent, prepare EDTA [M] 0.5 millimolar (mM) as follows:

1. Dilute 1 to 1000 EDTA [M] 0.5 Molarity (M) in distilled water; e.g. 5 mL EDTA [M] 0.5 Molarity (M) in 995 mL distilled water.
2. Filter sterile using 0.22 µm filter.
3. Store at RT.

Preparation of destination vessel

5m

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

2 Aspirate and discard coating solution from wells of destination vessel.

3 Add hPSC maintenance media per destination vessel, refer to **Table 1** for recommended volumes.

5m

A	B	C	D	E
Culture Vessel	DPBS (mL)	non-enzymatic reagent (mL)	Media for hPSC harvesting (mL)	Media in destination vessel (mL)
12 WP (per well)	0.5	0.5	1.5	1
6 WP (per well)	1	1	2.5	2
T25	2	2	5	7
10 cm	3	3	7.5	10
T75	5	5	10	15

Table 1. Recommended volumes according to vessel format

4 Keep prepared destination vessel at 37 °C until usage.

hPSC non-enzymatic passaging

51m

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

30m

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.

6 Aspirate and discard media from selected source vessel. 1m

7 Wash the well once using DPBS (no Calcium/no Magnesium). 2m

8 Add enzyme-free reagent, refer to **Table 1** for recommended working volumes. 1m

9 Incubate the vessel at 37 °C monitoring hPSC detachment using a microscope. Refer to **Table 2** for recommended incubation times. 5m

Cells will be ready to harvest when colonies start to be loosen though still attached.

	A	B	C	D	E
	Reagent	EDTA 0.5mM	GDR	ReLeSR	Versene
	Time (min)	2-3	2-3	5-7	2-5

Table 2. Recommended incubation times according to enzyme-free reagent.

Note

This incubation can also be done at Room temperature, recommendation at 37 °C is to avoid hPSC stress.

10 Aspirate and discard enzyme-free reagent. 1m

11 Add fresh hPSC maintenance media to the wells, refer to **Table 1** for recommended harvesting volumes. 2m

12 Pipette up and down using 5 mL serological pipette against the bottom of the well to dislodge cells from the culture surface. 3m

Note

Repeated execution of this step leads to progressively smaller hPSC aggregates, which can increase cellular stress and promote cell death. Aim to maintain a balance that results in a uniform aggregate suspension - ideally with sizes ranging from approximately 50-200 µm while minimizing shear stress.

Note

The use of scrapers is generally not recommended. However, for hPSC cultures with strong adherence properties, employing a scraper may be preferable to prolonged incubation with enzyme-free dissociation reagents.

13 Collect hPSC aggregates suspension using 5 mL pipette and directly distribute drop-wise the approximate volume for splitting ratios: 1/10, 1/15, 1/20 (or as desired) to the destination vessel/s. 5m

Note

Alternatively, aggregates suspension can be transferred to a conical tube prior distribution to better assess suspension volume.

14 Gently move destination vessels with freshly passed aggregates in cross shape to distribute them evenly. 1m

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

16 After 24:00:00 perform full media change. For further hPSC culture refer to protocol **Maintenance of hPSC** and protocol **Reference pictures of hPSC cultured in defined conditions**