May 23, 2025

Thawing of cryopreserved hPSC

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.e6nvwd282lmk/v1

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...

DOI: dx.doi.org/10.17504/protocols.io.e6nvwd282lmk/v1

Protocol Citation: Valeria Fernandez Vallone, Lyn Healy, Nathalie Lefort, Katarzyna Ludwik, Tamer Onder, Lisa Pavinato, Fatma Visal FVO OKUR, Harald Stachelscheid 2025. Thawing of cryopreserved hPSC. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.e6nvwd282lmk/v1

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

Created: November 21, 2023

Last Modified: May 23, 2025

Protocol Integer ID: 91286

Keywords: hPSC, thawing, cryopreservation, iPSC

Funders Acknowledgements:
COST Action CorEuStem
Grant ID: CA20140

Abstract

This protocol describes thawing of hPSC.

Guidelines

Thawing procedure is described for one cryovial. The protocol description is general and applies to multiple hPSC lines frozen as clumps or single cells.

It is recommended to thaw hPSC in same culture conditions that were used before freezing.

Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 2/5/10 mL pipettes
- 15/50 mL conical tubes
- 10/200/1000µL tips and pipettes (optional)
- Cell culture treated plastic vessels of choice e.g. 24, 12 or 6-well plates
- Cell counting equipment
- Aspirator pump with disposable pipette
- Centrifuge
- Microscope
- Water/bead warming bath
- Class II Biosafety Cabinet
- Incubator at 37^{o}C and 5% CO_2 or under hypoxic conditions, 5% O_2/ 5% CO_2

MEDIA AND REAGENTS

hPSC culture conditions and survival factors choice depend on hPSC line and individual lab practices. For options refer to protocols:

Coating of tissue Culture Vessels for hPSC

Maintenance of hPSC

Survival factors for hPSC growth

Safety warnings

hPSC culture media supplementation with survival factors is required for hPSC survival upon thawing.

Preparation of destination vessel

35m

1 Prepare coated tissue culture vessels according to hPSC line requierements and desired format. For coating procedure refer to protocol **Coating of tissue Culture Vessels for hPSC** 5m

Note

It is recommended to thaw hPSC on the same matrix that was used before freezing.

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

hPSC thawing

43m

3 Prepare required volume of the reagents and media supplemented with survival factors according to **Table 1.** Additionally prepare 10 mL of media for **Step 8**. Equilibrate the media to Room temperature or 37 °C . 10m

	A	F
	Culture vessel	hPSC media + survival factor (final: mL/per well
	24 well	0.5
	12 well	1
	6 well	2
	T25	7

Table 1. Recommended volumes according to vessel format

Note

hPSC culture media: it is recommended to thaw hPSC in same media used before freezing.
For options refer to protocol **Maintenance of hPSC**. For thawing media has to be supplemented with Survival factors. For options refer to protocol **Survival factors for hPSC growth**.

4 Remove cryovial containing frozen hPSC from liquid N_2. 5m

5 Sanitize the tube with [M] 70 % volume ethanol. 2m

6 Thaw hPSC suspension at 37 °C using water or bead bath for about 00:02:00 - 00:04:00 leaving small amount of un-thawed media. 6m

Note

If using water bath, carefully swirl vial in the water, avoid immersing the vial above the level of the cap.

7 Using 2 mL pipette transfer slowly hPSC suspension from the cryovial to a 15 mL conical tube. 3m

8 Dropwise add 10 mL of culture media supplemented with survival factors to hPSC suspension while gently swirling the tube, refer to **Table 1** for recommended volumes. 1m

9 Centrifuge hPSC suspension at 300 x g, 00:05:00 5m

10 Aspirate and remove the supernatant without disrupting the cell pellet. 1m

11 Using a 5 mL pipette gently add 2 mL of hPSC culture medium supplemented with survival factors to the pellet. 1m

12 Gently resuspend the hPSC pellet by pipetting up and down. 2m

Note

After homogeneous re-suspension and if hPSC were frozen as single cells, cell counting and viability assessment using e.g. Trypan Blue 0.4%. solution can be performed.

13 Aspirate coating solution from the destination vessel. 30s

14 Seed hPSC at desired density according to well size/surface. 5m

15 Rock the plate side to side, back and forth to spread the cells across the well. 1m

16 Incubate the vessels at 37 °C and at [M] 5 % volume CO_2. 30s

17 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**