May 23, 2025

Freezing of hPSC

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.rm7vzx278gx1/v1

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Protocol Citation: Valeria Fernandez Vallone, Lyn Healy, Nathalie Lefort, Katarzyna Ludwik, Tamer Onder, Lisa Pavinato, Fatma Visal FVO OKUR, Harald Stachelscheid 2025. Freezing of hPSC. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.rm7vzx278gx1/v1

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

Created: November 21, 2023

Last Modified: May 23, 2025

Protocol Integer ID: 91274

Keywords: cryopreservation, freezing, hPSC, iPSC, Bambanker

Funders Acknowledgements:
COST Action CorEuStem
Grant ID: CA20140

Abstract

This protocol describes cryopreservation of hPSC as clumps or single cells.

Guidelines

hPSC culture quality is highly relevant for a successful freezing/thawing procedure.
We recommend freezing cultures at ~70-80% confluency. High quality cultures show spontaneous differentiation below 5-10% of cultured surface.

Materials

LABORATORY EQUIPMENT AND CONSUMABLES

Use sterile material

- 1/5/10 mL serological pipettes
- 15/50 mL conical tubes
- 10/200/1000µL tips and micropipettes (optional)
- 1 or 1.5 mL cryo-vials
- Cell scraper
- Cell counting equipment
- Aspirator pump with disposable pipette
- Centrifuge
- Microscope, if available Stereo Microscope
- Freezing container (Mr. Frosty) filled with 100% 2-propanol (pre-chilled), alternatively equipment for automated controlled freezing.
- Class II Biosafety Cabinet

MEDIA AND REAGENTS

Bambanker™ 1×120mL **Nippon Genetics Catalog #**BB01

STEM-CELLBANKER - GMP Grade **amsbio Catalog #**11890

Cryostar CS10 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C2874

Alternatively, fetal bovine serum 90% / DMSO 10% solution. However, this freezing medium is less defined and not xeno-free.

Protocol materials

BAMBANKER **BioCat GmbH Catalog #**BB03-NP

Bambanker™ 1×120mL **Nippon Genetics Catalog #**BB01

STEM-CELLBANKER - GMP Grade **amsbio Catalog #**11890

Cryostar CS10 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C2874

Before start

Prepare labelled or barcoded cryo-vials.

Preparation

25m

1 Visually inspect hPSC culture using a microscope. Culture ready for freezing should be of good quality (see Guidelines section). 10m

2 Refer to **Table 1** to prepare adequate amount of dissociation reagent and freezing medium according to the culture vessel format and amount of vessels/wells to be frozen. 15m

A	B	C	D
Culture vessel	Dissociation reagent [mL]	Cryopreservation media [mL]	Re-suspension media [mL]
24 well	0.25	0.25	0.5
12 well	0.5	0.5	1
6 well	1	1	2
T25	3	3	6
T75	6	10	10
10 cm dish	3	10	10

Table 1. Recommended volume according to vessel format

Note

Freezing as single cells additionally requires the use of re-suspension media (culture media supplemented with survival factors as described in protocol: **Survival factors for hPSC growth**) to wash out enzymatic dissociation reagent.

Note

Dissociation reagent will depend on the freezing method choice: as clumps or as single cells see STEP CASE.

Freezing medium options are described in material and methods, we recommend the use of BAMBANKER **BioCat GmbH Catalog #**BB03-NP

3 Choose freezing method:

STEP CASE

hPSC freezing as clumps 10 steps

Clump freezing

1d 0h 16m

4 Aspirate and discard medium from the vessel. 1m

5 Rinse once with required volume of non-enzymatic dissociation reagent, refer to **Table 1** for recommended volumes. 2m

Note

A variety of non-enzymatic dissociation reagents can be used. For options refer to protocol:
Non-enzymatic passaging of hPSC

6 Add required volume of non-enzymatic dissociation reagent to the culture vessel, refer to **Table 1** for recommended volumes. 1m

7 Incubate for 00:03:00 - 00:05:00 at Room temperature . 5m

Note

Monitor under microscope. When hPSC colonies look losen, stop the incubation.

8 Gently aspirate non-enzymatic dissociation reagent gently without disturbing the cells and discard. 1m

9 Add required volume of freezing medium to the culture vessel, refer to **Table 1** for recommended volumes. 1m

10 Gently tap the plate 00:00:05 - 00:00:10 to dislodge the cells from the plastic surface. 1m

Note

Gently scraping the vessel surface aids in clump harvesting when plate tapping alone is insufficient.

11 Use a 2 mL pipette to transfer hPSC suspension (ideally 1 mL) into pre-labeled cryo-vials. 1m

Note

If multiple wells or vessels from same culture are going to be frozen, hPSC suspensions have to be
pooled and homogenize in a 15 or 50 mL tube before freezing. Thus, every cryo-vial contains equal material.

12 Immediately place cryo-vials into the freezing container and place at -80 °C 3m
Overnight

Note

When available, controlled freezing using automated devices e.g. Viafreeze (Cytiva) is recommended.

13 Transfer hPSC containing cryo-vials to a liquid N_2 tank after 24:00:00 1d