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Freezing of hPSC

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We use this protocol and it's working

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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 N₂ tank after  24:00:00

1d