[Protocol 1.01.1] Freezing of iPSCs using Bambanker freezing medium

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No tags associated

Created: 11.08.2022 12:06
Last modified: 07.09.2022 14:28

No custom dates added

Protocol 1.01.1 Freezing of iPSCs using Bambanker freezing medium
Version: 1.0 (11.08.2022)

Media and Reagents:
BambankerTM (Nippon Genetics, BB01)
0,5 M EDTA (Thermo Fisher, 15575-020)

Materials and Equipment:
Aspiration Pump
Cell scraper
Freezing container (Mr. Frosty) filled with100% 2-propanol (pre-chilled)
Pre-labeled Cryo vials (NUNC, 479-6843)

1. Introduction and Purpose

This protocol describes the methodology for cryopreservation of induced pluripotent stem cells (iPSCs) using BambankerTM freezing medium.

2. Procedure Cryopreservation
1. Cells must be cryopreserved when they are in their log phase of growth (60 - 70 % confluence).
2. Use a microscope to visually identify regions of differentiation and remove regions of differentiation by scraping with a pipette tip. This selection should not exceed 20% of the well if the culture is of high quality.
3. Aspirate medium from the culture and rinse once with appropriate volume of PBS (see table below).

Culture Vessel	Surface Area [cm2]*	Volume 0.5 mM EDTA [ml]	Volume Bambanker TM[ml]
24 well plate	2	0.25	0.25
12 well plate	4	0.5	0.5
6 well plate	10	1	1
T25 flask	25	3	3
T75 flask	75	6	6

*Exact sizes are manufacturer/type dependent

4. To lift cells from the tissue culture vessel add appropriate volume of 0.5 mM EDTA to the culture (see table above) and incubate for 3 - 5 minutes at RT.
Note: Alternatively the incubation can occur at 37°C on heating plate in the IVF bench. In this case the incubation time will be reduced to 2 - 3 minutes.
5. Check under the stereo microscope, if cell colonies start to show holes throughout. Care should be taken not to incubate until the colonies detach from the plate.
6. Aspirate EDTA gently and add appropriate volume of Bambanker TM freezing medium (see table above) to the cells.
7. Gently tap the plate 5 - 10 seconds to dislodge the cells from the plastic and use a 5 ml pipette to fill the cell suspension (ideally 1 ml) into labeled cryo vials. Do not aspirate more than 2 times to avoid breaking the cell clumps into single cells.
Note: Alternatively the cells can also be scraped off with a cell scraper. Take care to keep the clumps as big as possible!
8. Place the cryo vials immediately into the freezing container and place at -80°C overnight.
9. Transfer the cells to a liquid N2 tank the next day.

Appendix
General points to consider:
- Wells are 60 - 70% confluent at time of cryopreservation.
- Cryopreservation should be done approximately 1 day before the cells are ready to passage.
- Before cryopreservation, pluripotent stem cells should be of high quality (primarily undifferentiated with less than 20% of the cells being differentiated).
- Pluripotent stem cells will have improved survival following thawing if cryopreserved as large clumps.
- 1 ml of cryopreservation media should be used for every well of a 6-well plate being frozen.However, if the wells are at low density (less than 50% confluent), 1 ml of cryopreservation media may be used for every 2 wells.

Label of cryo vials as follows and place on ice:
- Cell type/cell line name
- Passage
- Freeze date
- Your Initials

Relevant applicable documents:
Protocol 1.01.2 Thawing of hPSC from BambankerTM freezing medium