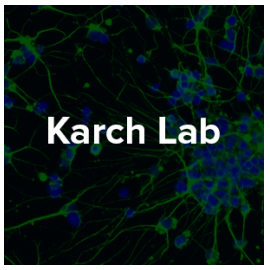
Feb 27, 2019

iPSC Freezing



In 1 collection

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Neurodegeneration Method Development Community
Tech. support email: **ndcn-help@chanzuckerberg.com**

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

Created: February 18, 2019

Last Modified: February 27, 2019

Protocol Integer ID: 20473

Guidelines

This protocols is part of the **Screening Edited iPSC Clones collection**.

Materials

STEP MATERIALS

Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher Scientific** **Catalog #**A1110501

Protocol materials

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Safety warnings

Please refer to the SDS (Safety Data Sheet) for information about hazards, and to obtain advice on safety precautions.

1 Aspirate media

2 Gently wash cells with 1x PBS.

Note

Use 2-3 mL per well in 6 well plate.

3 Add Accutase (Gibco A11105-01) directly to the cells and incubate at 37 °C for 3-4 minutes. 00:03:00

Note

Individual donor cell lines exhibit variable sensitivity to accutase-mediated dissociation. Thus, monitor cells closely to determine when single cell dissociation is achieved.

Note

For a 6 well plate, add 0.75-1 mL per well.
For a 10cm^2 dish, add 3 mL.

Accutase™ Cell Dissociation Reagent **Gibco - Thermo Fisher Scientific Catalog #**A1110501

4 Tap dish to aid in dislocation of cells.

5 Add DMEM/F12 directly to cells.

Note

- For a 6 well plate, add 2 mL - 4 mL per well.
- For a 10cm^2 dish, add 9 mL
- If cells remain attached, use a cell scraper to gently dislodge cells (apply gentle pressure and use 1-2 passes to remove cells)

6 Collect cells in conical tube (15mL/50mL depending on volume).

7 Add 2 mL - 5 mL DMEM/F12 to dish to remove all cells from the dish and add to conical tube.

8 Centrifuge cells at 750 rpm for 00:03:00 at room temperature.

9 Carefully aspirate supernatant.

Note

To avoid aspirating cell pellet, it is OK to leave a small amount of media (0.5-1mL).

10 Resuspend cell pellet with mTesR1 (No Rock Inhibitor).

- Use volume appropriate for freezing.
- Assume 1 mL per cryovial total and add ½ total volume of mTesR1.
- Pipet cells 1-2 times only to preserve cell clumps.

Note

Example: to freeze 10 tubes, you will need 10 mL total and will add 5 mL mTesR1 to cell pellet (and 5 mL of 2x Freezing Media below).

11 Add an equal volume of cold 2x Freezing Media (20% DMSO, FBS). Pipet cells 1 time only to preserve cell clumps.

12 Transfer cell suspension to pre-labeled cryovials (1 mL per cryovial).
- Ensure that cryovials are labeled with the following
  -Cell Type
  -Line Name
  -Passage #
  -Date
  -Your Name

13 Freeze vials at -80 °C in foam racks for 48-72 hours. 48:00:00

14 Transfer vials to liquid nitrogen for long-term storage.