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Working

iPSC Freezing

In 1 collection

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Neurodegeneration Method Development Community

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

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

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Accutase™ Cell Dissociation Reagent	A1110501	Gibco - Thermo Fischer

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.

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

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

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

Accutase™ Cell Dissociation Reagent
by Gibco - Thermo Fischer
Catalog #: A1110501

4 Tap dish to aid in dislocation of cells.

5 Add DMEM/F12 directly to cells.

- 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.

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.

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.