



Feb 27, 2019 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

Accutase™ Cell Dissociation Reagent

A1110501

CATALOG #

VENDOR

Gibco - Thermo Fischer

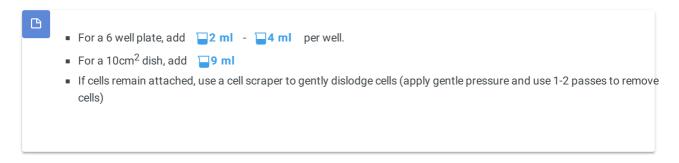
SAFETY WARNINGS

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

- Aspirate media
- 9 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 10cm² dish, add 3 mL.



- 4 Tap dish to aid in dislocation of cells.
- 5 Add DMEM/F12 directly to cells.



- 6 Collect cells in conical tube (15mL/50mL depending on volume).
- 7 Add 22 ml 35 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.



- 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 8-80 °C in foam racks for 48-72 hours. (348:00:00
- 14 Transfer vials to liquid nitrogen for long-term storage.

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