Preparing hPSC-derived neurons for single-cell RNA sequencing

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bpcbmisn

Neurodegeneration Method Development Community
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ABSTRACT

This protocol is about Preparing hPSC-derived neurons for single-cell RNA sequencing.

ATTACHMENTS

Preparing_hPSC-derived_neurons_for_single-cell_RNA_sequencing.pdf

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ATTACHMENTS

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MATERIALS TEXT

Materials

☒ Actinomycin D **Sigma Aldrich Catalog #A1410-10MG**

☒ Bovine Serum Albumin (BSA) **Sigma Aldrich Catalog #A0281**

☒ DMEM/F-12, GlutaMAX™ Supplement **Thermo Fisher Catalog #31331028**

☒ DNase Vial (D2) **Worthington Biochemical Corporation Catalog #LK003170**

☒ DPBS, no calcium, no magnesium **Thermo Fisher Catalog #14190144**

☒ Eppendorf® LoBind microcentrifuge tubes **Sigma Aldrich Catalog #Z666505-100EA**

☒ Falcon 40 µm Cell Strainer **Corning Catalog #352340**

☒ PDS Kit Papain Vial **Worthington Biochemical Corporation Catalog #LK003176**

☒ Trypan Blue Stain (0.4%) for use with the Countess™ Automated Cell Counter **Thermo Fisher Scientific Catalog #T10282**

☒ TrypLE™ Select Enzyme (10X), no phenol red **Thermo Fisher Catalog #A1217701**

☒ Y-27632 dihydrochloride (Rock Inhibitor) **Contributed by users Catalog #DNSK-KI-15-02**

Media and Reagents

Dissociation Solution:

Name	Volume
DPBS	2.5 mL
TryPLE	2.5 mL
Papain	1 mL
Actinomycin D (30 mM stock)	2.5 µL

Wash Buffer 1:

Name	Volume
DMEM/F-12	15 mL
Y-27632 (10 mM stock)	15 µL
Dnase I (2 mg/mL stock; 5990 U/mg)	250 µL
Actinomycin D (30 mM stock)	15 µL

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Wash Buffer 2:

Name	Volume
DPBS	10 mL
BSA in DPBS (4% stock)	100 µL
Actinomycin D (30 mM stock)	10 µL

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

ABSTRACT

This protocol is about Preparing hPSC-derived neurons for single-cell RNA sequencing.

BEFORE STARTING

Prepare Media and Reagents as described in section 'Materials'.

1 Prepare dissociation solution and wash buffers.

2

Wash cells 2x with DPBS.

3

Add dissociation solution (**1 mL** for 6 well plate, **250 µl** for 24 well plate).

4 12m

Incubate at **37 °C** for up to **00:10:00** until cells are ready to detach easily (test by pipetting dissociation solution onto cells with P1000, check every **00:02:00**).

5

Detach in **1 mL Wash Buffer 1** and break up by gently pipetting up and down within the well until there are no visible clumps (triturate for ~20x).

6 Pass cells through a 40 µm cell strainer and transfer to a Lobind Eppendorf.

7

Pellet at **200 x g, Room temperature , 00:03:00** .

8

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Aspirate media and wash pellet 2x with **1 mL Wash Buffer 2** (spin at **200 x g, Room temperature , 00:03:00**).

9 Pass cells through 40 µm cell strainer into Lobind Eppendorf.

10 Dilute 1:20 in **200 µl Wash Buffer 2** and count using Trypan blue and Countess (take average of 3x counts).

Note: If using NucleoCounter, ensure that there is no more than 3% aggregation. If >3%, pass through cell strainer again.

11 Dilute to 4.26 x10^5 cells per mL and take **47 µl cell suspension** **On ice** to CRUK for 10X library prep (47 µL at this density equates to 20,000 cells).

Notes

- To target 10,000 cells -> 47 µL of 2.13 x 10^5 cells per mL
- For Novaseq V3, up to 25,000 cells can be tolerated, but this is not recommended as there will be excess doublets

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