Preparing hPSC-derived neurons for single-cell RNA sequencing

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 We use this protocol and it's working

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Preparing hPSC-derived neurons for single-cell RNA sequencing

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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](#)

MATERIALS

Materials

- ⊗ Actinomycin D **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A1410-10MG**
- ⊗ Bovine Serum Albumin (BSA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0281**
- ⊗ DMEM/F-12, GlutaMAX™ Supplement **Thermo Fisher Catalog #31331028**
- ⊗ DNase Vial (D2) **Worthington Biochemical Corporation Catalog #LK00317**
- ⊗ DPBS, no calcium, no magnesium **Thermo Fisher Catalog #14190144**
- ⊗ Eppendorf® LoBind microcentrifuge tubes **Merck MilliporeSigma (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

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).

BEFORE START INSTRUCTIONS

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 μ L for 24 well plate).

4 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).

12m

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 μ m cell strainer and transfer to a Lobind Eppendorf.

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

8 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

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

11 Dilute to 4.26×10^5 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).

Note

Notes

- To target 10,000 cells $\rightarrow$ 47 μL of 2.13×10^5 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