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Culturing and passaging of iPSC derived intestinal organoids

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

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Disclaimer

Protocol Particulars Video

The video below is a supplement with extra context and tips, as part of the Aligning Science Across Parkinson's (ASAP) Protocol Particulars video interview series, featuring conversations with protocol authors.

https://www.youtube.com/embed/lA1YeJurpug?si=nb3HPUyzMtBiAKq4

Abstract

Culturing and passaging of iPSC derived intestinal organoids derived using STEMDIFF intestinal organoid kit. We usually use organoids after 5 passages once consistent growth has been established and until 15th passage.

Materials

Intestinal growth medium.

DMEM F12/HEPES **Gibco - Thermo Fischer** **Catalog #**113300

N-2 max supplement **R&D Systems** **Catalog #**AR009

N21-MAX Media Supplement (50X) **R&D Systems** **Catalog #**AR008

HEPES **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H6147

FC WNT

EGF

Noggin

SB202

Lacetylecysteine

Nicotinamide

L-Gastrin

A3801

Protocol materials

- N-2 max supplement **R&D Systems** **Catalog #**AR009
- N21-MAX Media Supplement (50X) **R&D Systems** **Catalog #**AR008
- HEPES **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H6147
- DMEM F12/HEPES **Gibco - Thermo Fisher Scientific** **Catalog #**113300
- STEMdiff intestinal organoid kit **STEMCELL Technologies Inc.** **Catalog #**05140
- DMEM F12/HEPES **Gibco - Thermo Fisher Scientific** **Catalog #**113300
- DMEM F12/HEPES **Gibco - Thermo Fisher Scientific** **Catalog #**113300
- Anti-Adherence Rinsing Solution **STEMCELL Technologies Inc.** **Catalog #**07010
- ACCUTASE™ 100 mL **STEMCELL Technologies Inc.** **Catalog #**7920
- DMEM F12/HEPES **Gibco - Thermo Fisher Scientific** **Catalog #**113300
- Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**3445-001-01
- Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**3445-001-01
- Nunc™ Cell-Culture Treated Multidishes, 12 well **Thermo Fisher** **Catalog #**150628
- DMEM F12/HEPES **Gibco - Thermo Fisher Scientific** **Catalog #**113300
- Anti-Adherence Rinsing Solution **STEMCELL Technologies Inc.** **Catalog #**07010
- Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**3445-001-01
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- Nunc™ Cell-Culture Treated Multidishes, 12 well **Thermo Fisher** **Catalog #**150628

Establishing organoids.

1 Intestinal organoids were generated using
STEMdiff intestinal organoid kit **STEMCELL Technologies Inc. Catalog #**05140
until protocol stage 6.2.2.4. For some cell lines yields of organoids can be very low using STEMcell methods. We usually harvest organoids at 9 days differentiation.

2 Prepare required number of 15mL falcon by washing with
Anti-Adherence Rinsing Solution **STEMCELL Technologies Inc. Catalog #**07010 .
Rinse with PBS.

3 Thaw an aliquot of
Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**3445-001-01
on ice. 60 µL will be required for each well.

4 Monolayer cultures in 24 well plate displaying spheroid budding are washed 3 times with cold DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 to remove spheroids and place in precoated falcon.

4.1 if yield is low add 1 mL
ACCUTASE™ 100 mL **STEMCELL Technologies Inc. Catalog #**7920 to monolayer for 00:02:00 at 37 °C . Remove detached monolayer in accutase and add to falcon.
2m

5 Make volume up 10 mL with
DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 and allow organoids and fragments of tissue to sink

6 Carefully aspirate supernatant leaving 1 mL behind in falcon containing organoids. Add a new 1ml of DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 to the spheroids. Centrifuge 300 x g, 4°C, 00:05:00 .
5m

7 Carefully remove as much supernatant as possible. Place falcon on ice and add 60 µL Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**3445-001-01 for each well required. Recommend plating 100-200 domes per dome (2 domes per well).

8 Gently mix organoids into the Cultrex being careful not to introduce bubbles. Using a new pipette tip pick up 30 µL of mix and transfer to one well of a Nunc™ Cell-Culture Treated Multidishes, 12 well **Thermo Fisher Catalog #**150628 . Repeat the process so that the well contains 2 domes. Allow domes to solidify at 37 °C in incubator for 00:30:00 . We find organoids distribute best if turn the plate upside down. We have found that nunc plates provide the best adherence for domes without detachment over the 10 day growth period. 30m

9 Carefully add 1 mL of Intestinal organoid growth medium. Incubate at 37 °C . Feed cells every 2/3 days.

Passaging intestinal organoids.

10 Coat a 15ml Falcon with Anti-Adherence Rinsing Solution **STEMCELL Technologies Inc. Catalog #**07010 before washing off with PBS.

11 Thaw an aliquot of Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**3445-001-01 ready for passaging. Usually split a well from a 12 well plate 1:4 and allow 60 µL for each new well.

12 After 10 days in culture domes are ready for passaging. Remove medium from well. Add 1 mL of cold DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 . Gently break up the pellet and until it comes loose from the plate. Add into prepared falcon tube. Combine up to 4 wells from 12 well plate.

13 Wash well with a further 1 mL DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 to collect any remaining organoids and add to falcon. Make up to 10 mL .

14 Keep falcon on ice for 5 minutes to allow organoids to sink. Carefully remove supernatant leaving 1 mL in falcon with organoids. Add a new 2 mL of DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 . Using a1ml pipettor pipette suspension up and down 5 times to gently break apart the domes. incubate on ice for 00:05:00 . 5m

15 Centrifuge 300 x g, 4°C, 00:05:00 . Carefully aspirate supernatant. Cultrex will form a cloudy layer on pellet at bottom of tube. try to remove as much of this as possible to clear single cells and smaller fragments. Add 2 mL DMEM F12/HEPES **Gibco - Thermo Fischer Catalog #**113300 to pellet. Using a 1ml pipette vigorously pipette organoids up and down 20-25 times. Incubate on ice for 00:05:00 10m

16 Centrifuge pellet 300 x g, 4°C, 00:05:00 . Carefully remove as much supernatant as possible. Place falcon on ice and add 60 µL Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear® **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**3445-001-01 per 12 well required. Carefully mix organoid fragments with cultrex ensuring not to introduce bubbles. Split into two 30 µL domes per well of Nunc™ Cell-Culture Treated Multidishes, 12 well **Thermo Fisher Catalog #**150628 . Incubate at 37 °C for 00:30:00 . 35m

17 Add 1 mL of intestinal organoid growth medium per well.

Intestinal organoid growth medium

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