

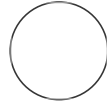


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Culturing and passaging of iPSC derived intestinal organoids

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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

Lacetylcysteine

Nicotinamide

L-Gastrin

A3801

OPEN ACCESS



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















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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 μ L  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 μ L 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.
- 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 μ L 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  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  . Using a 1ml 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  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  per 12 well required. Carefully mix organoid fragments with cultrex ensuring not to introduce bubbles. Split into two  30 µL domes per well of  . Incubate at  37 °C for  00:30:00 . 35m
- 17 Add  1 mL of intestinal organoid growth medium per well.

Intestinal organoid growth medium

