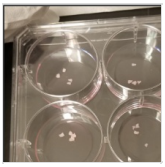

Sep 10, 2020

Splitting p0 (6wp) to p1 (T75)

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.8gjhtun

Neurodegeneration Method Development Community
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ABSTRACT

Protocol includes splitting outgrowth of dural cell from a 6 well plate into one T75 flask for expansion.

DOI

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PROTOCOL CITATION

Andrea Argouarch 2020. Splitting p0 (6wp) to p1 (T75). **protocols.io** https://dx.doi.org/10.17504/protocols.io.8gjhtun

COLLECTIONS

Dural Cell Isolation and Culturing - Collection

CREATED

Oct 19, 2019

LAST MODIFIED

Sep 10, 2020

PROTOCOL INTEGER ID

28907

PARENT PROTOCOLS

Part of collection
Dural Cell Isolation and Culturing - Collection

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Fetal Bovine Serum	97068-091	Vwr
Trypsin-EDTA (0.05%), phenol red	25300062	Thermo Fisher
Dumont #5 Forceps	11251-30	Fine Science Tools
GeneMate Cell Scrapers & Lifter	490000-254	Vwr
DPBS, no calcium, no magnesium	14190250	Thermo Fisher
DMEM, high glucose, pyruvate	11995073	Thermo Fisher

NAME	CATALOG #	VENDOR
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher

Observations

1 After 3 weeks, split 6 wells of the 6 well plate (6wx6wp) into 1xT75 flask
a. Keep 6wp as backup by re-feeding the entire plate after trypsinization.

2 Cells will grow on the plastic and maybe on the glass coverslip itself

Preparation

3 Turn off UV lights and clean hood with 70% ethanol

4 Clean items with 70% ethanol and bring into hood
a. Autoclaved # 5 Forceps

Dumont #5 Forceps
by Fine Science Tools
Catalog #: 11251-30

b. 10 cm dish for forceps, cell scraper, discarded coverslips, and discarded tissue
c. Sterile cell scraper

GeneMate Cell Scrapers & Lifter
by Vwr
Catalog #: 490000-254

d. DPBS $^{-/-}$

DPBS, no calcium, no magnesium
by Thermo Fisher
Catalog #: 14190250

e. Sterile Filtered Media with PenStrep

DMEM, high glucose, pyruvate
by Thermo Fisher
Catalog #: 11995073

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Penicillin-Streptomycin
by Gibco - Thermo Fisher
Catalog #: 15140122

Fetal Bovine Serum
by Vwr
Catalog #: 97068-091

f. Label 1xT75 flask with ID, date, and p1, **add** 15 mls **15 mL** of media per flask

g. 0.05% Trypsin

Trypsin-EDTA (0.05%), phenol red
by Thermo Fisher
Catalog #: 25300062

i. Aliquot and warm before use

Culturing

5 Aspirate old media

6 Rinse by adding 3 mls **3 mL** of DPBS per well

7 Lift coverslips with forceps to allow adequate washing under the coverslip and gently swirl

8 Aspirate DPBS

9 Add 2 ml **2 mL** of trypsin per well and lift coverslips breifly with forceps

10 Place in incubator **37 °C** for ~5 mins **00:05:00** until cells start to detach

a. Can also gently tap the side of the plate to detach cells from the coverslip

11 Add 2 mls **2 mL** of media per well to inactivate trypsin

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12 With forceps, flip over coverslip within each well
a. Can remove tissue and place in 10 cm dish to discard

13 Use cell scraper to gently scrap off cells in each well attached to the coverslip

14 Discard each coverslip in the 10 cm dish

15 Use cell scraper to scrap off remaining cells (cells that are attached to bottom of plate)

16 Check under microscope and mark areas with cells still attached with a pen

17 Re-scrape cell gently if needed

18 Collect cell suspension in a 50 ml conical

19 Rinse wells with additional media ~6mls **6 mL** to collect any remaining cells

20 Spin at 1000 rpm **1000 rpm** for 5 mins **00:05:00**

21 Re-feed old 6wp plate for backup, 2 mls **2 mL** per well

22 Aspirate supernatant, being careful to not aspirate the pellet

23 Tap the pellet to resuspend

24 Add media to a final volume of 5 mls **5 mL** and pipette up and down to mix

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25 Add cell suspension to flask

26 Place flask in incubator (5% CO2, 37^{O}C) **37 °C**

27 Observe and feed the next day to remove debris, then feed every 2-3 days until 90-100% confluent

Clean Up

28 Throw away biohazard materials properly
a. 10 cm dishes with glass coverslips should be thrown away in biohazard red sharp container

29 Clean surgical tools, wear waterproof lab coat and eye protection/PPE
a. Brush and clean with 409 soap water. Rinse with water, dry on kimwipe, rinse with 100% ethanol, and then dry completely with kimwipe to prevent rust or water marks.
b. Prep for next autoclaving cycle

30 Clean and sterilize hood with 70% ethanol and turn on UV

31 Update cell culture notes in lab notebook

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