Sep 10, 2020

Splitting p1 (1xT75) to p2 (2xT150)

In 1 collection

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

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ABSTRACT

Protocol includes splitting growing cell line from a T75 flask into two T150 flasks for expansion.

DOI

dx.doi.org/10.17504/protocols.io.8gnhtve

PROTOCOL CITATION

Andrea Argouarch 2020. Splitting p1 (1xT75) to p2 (2xT150). **protocols.io** https://dx.doi.org/10.17504/protocols.io.8gnhtve

COLLECTIONS

Dural Cell Isolation and Culturing - Collection

CREATED

Oct 19, 2019

LAST MODIFIED

Sep 10, 2020

PROTOCOL INTEGER ID

28910

PARENT PROTOCOLS

Part of collection
Dural Cell Isolation and Culturing - Collection

STEPS MATERIALS

NAME	CATALOG #	VENDOR
DPBS, no calcium, no magnesium	14190250	Thermo Fisher
DMEM, high glucose, pyruvate	11995073	Thermo Fisher
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
Fetal Bovine Serum	97068-091	Vwr
Trypsin-EDTA (0.05%), phenol red	25300062	Thermo Fisher

Observations

1 At 90-100% confluency, split T75 flask into 2xT150 flasks

Preparation

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

3 Clean items with 70% ethanol and bring into hood

a. DPBS -/-

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

b. Sterile Filtered Media

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

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

Fetal Bovine Serum
by Vwr
Catalog #: 97068-091

c. Label 2xT150 flasks with ID, date, and p2, add 28 mls **28 mL** of media per flask

d. 0.05% Trypsin

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

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Culturing

4 Aspirate old media

5 Rinse by adding 5 mls **5 mL** of DPBS per flask and gently swirling

6 Aspirate DPBS

7 Add 2 mls **2 mL** of trypsin per flask and place in incubator **37 °C** for 2-3 mins **00:03:00** until cells are stating to detach
a. Can also gently tap the side of the flask to detach cells

8 Check under microscope

9 Add 10 mls **10 mL** of fibroblast media per flask to inactivate trypsin

10 Collect cell suspension in a 15 ml conical

11 Spin at 600 rpm **600 rpm** for 4 mins **00:04:00**

12 Aspirate supernatant, being careful to not aspirate the pellet

13 Tap the pellet to resuspend

14 Add fibroblast media to 4 mls **4 mL**

15 Add 2 ml **2 mL** of cell suspension per flask

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

17 Observe and feed the next day, then feed every 2-3 days until 90-100% confluent

Clean Up

18 Throw away biohazard materials properly

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

20 Update cell culture notes in lab notebook

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