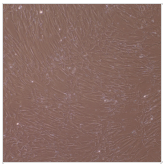

Sep 10, 2020

Dural Cell Isolation

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.8ghhtt6

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ABSTRACT

Isolation of cells from human dura mater. Protocol includes tissue freezing, cutting the tissue with surgical tools, plating into a 6 well plate, placing a coverslip on top of the tissue, and adding cell culture media.

DOI

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PROTOCOL CITATION

Andrea Argouarch 2020. Dural Cell Isolation . **protocols.io**
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COLLECTIONS

Dural Cell Isolation and Culturing - Collection

CREATED

Oct 18, 2019

LAST MODIFIED

Sep 10, 2020

PROTOCOL INTEGER ID

28905

PARENT PROTOCOLS

Part of collection
Dural Cell Isolation and Culturing - Collection

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Instant Sealing Sterilization Pouches, 3.5 x 5 in.	0181250	Thermo Fisher
25mm coverslips round	GG-25	
Dumont #5 Forceps	11251-30	Fine Science Tools
Fine Scissors - Tungsten Carbide	14568-12	Fine Science Tools
Instant Sealing Sterilization Pouches, 3.5 x 9 in.	0181251	Thermo Fisher

NAME	CATALOG #	VENDOR
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
Nalgene Cryogenic Vials	66008-706	Vwr
DMSO Bio-Max, Cell Culture Grade	40470005-2	bioworld
DPBS, no calcium, no magnesium	14190250	Thermo Fisher
DMEM, high glucose, pyruvate	11995073	Thermo Fisher
Fetal Bovine Serum	97068-091	Vwr

Preparation for Isolation

1 Prepare solid autoclaving

a. 25 mm coverslips – 7 coverslips per 13 cm pouch (slide coverslips in the middle of the pouch before opening)

25mm coverslips round
Catalog #: GG-25

b. Surgical Tools – 1 each per 23 cm pouch

i. Dumont # 5 Forceps

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

ii. Tungsten Scissors

Fine Scissors - Tungsten Carbide
by Fine Science Tools
Catalog #: 14568-12

c. Seal autoclave pouch and autoclave. Confirm that autoclave tape has turned black

Instant Sealing Sterilization Pouches, 3.5 x 9 in.
by Thermo Fisher
Catalog #: 0181251

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Instant Sealing Sterilization Pouches, 3.5 x 5 in.
by Thermo Fisher
Catalog #: 0181250

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

i. High Glucose DMEM with Sodium Pyruvate (1X)

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

ii. Heat Inactivated FBS (10%)

Fetal Bovine Serum
by Vwr
Catalog #: 97068-091

iii. PenStrep (1%)

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

c. Biopsy in 50ml conical with ~20 mls **20 mL** of media

d. 10 cm dish for biopsy and its media, labeled 'dirty'

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e. 6 well plate for washes, four wells labeled 1-4, **add** 3 mls **3 mL** of DPBS $^{-/-}$ each

f. 10 cm dish for rinsed biopsy, labeled 'clean', **add** ~15 mls **15 mL** of new fibroblast media. Make sure biopsy is submerged in media and will not dry out

g. 10 cm plate to hold tools labeled 'tools'

h. 6 well plate, labeled with ID, date, and p0

i. Autoclaved coverslips

j. Autoclaved surgical tools

k.3-4 Ln2 vials pre labeled for extra dural tissue

Nalgene Cryogenic Vials
by Vwr
Catalog #: 66008-706

i. Label: Date, ID, Dura Tissue

ii. Add 1 ml **1 mL** of freezing media per vial

l. Freezing media

i.100% DMSO to a final of 10%

DMSO Bio-Max, Cell Culture Grade
by bioworld
Catalog #: 40470005-2

ii. 90% of media

Isolation

4 Spray conical with biopsy into the hood and pour media with biopsy into the dirty 10 cm dish

5 With forceps, submerge biopsy in the 1st DPBS well, tilt, rinse and swirl for 1 min **00:01:00**

6 With forceps, submerge biopsy in the 2nd DPBS well, tilt, rinse and swirl for 1 min **00:01:00**

7 With forceps, submerge biopsy in the 3rd DPBS well, tilt, rinse and swirl for 1 min **00:01:00**

8 With forceps, submerge biopsy in the 4th DPBS well, tilt, rinse and swirl for 1 min **00:01:00**

9 With forceps, transfer the rinsed biopsy into the clean 10 cm dish with new media

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10 Cut biopsy into 3-5 regions, freeze 2-4 sections and keep 1 section to culture
a. Place pieces into ln2 vials with tweezers, careful not to touch the outside of the vial
c. Place in a Mr. Frosty at -80C **-80 °C** for 25-48hrs **48:00:00** , then transfer to ln2 **-190 °C** for long-term storage

11 Cut the small section of biopsy into smaller pinhead sized pieces with forceps and scissors
a. Don't let biopsy to dry out
b. Aim for 18-20 pieces depending on biopsy size
c. Once finished, cover dish and carefully move aside

12 Add 200 µl **200 µl** droplet of media in the center of each well of a 6 well plate
a. Place similar sized pieces of tissue within the middle of the droplet

13 Place a coverslip over each droplet, starting from one side, centering it, and dropping it into the well
a. Avoid air bubbles

14 Gently press the coverslip down to make pieces adhere evenly and firmly to the bottom of the well
a. Muse sure the coverslips are firm, secure, flat, even, and not wobbling
b. Keep the pieces in the center
c. Tissue should be bit squished out flat, but still dense and compact

15 Add 3 mls **3 mL** of media per well, making sure that the coverslips does not rise by gently placing the forceps on top of coverslip. Add the media quickly to break the surface tension

16 Observe tissue integrity under microscope

17 Gently place 6 well plate in incubator (5% CO2, 37^{O}C) **37 °C**

18 Let the biopsy tissue settle for a week before feeding, and observe cell growth. Do not move plate for the first week.

Clean Up

19 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

20 Aspirate biohazard media and throw away biohazard materials properly

21 Clean and sterilize hood with 70% ethanol and turn UV on. Update cell culture notes in lab notebook.

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