

Sep 10, 2020

Feeding 6wp

In 1 collection

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

Neurodegeneration Method Development Community

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ABSTRACT

Protocol for feeding dural cells after outgrowth from tissue in a 6 well plate.

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PROTOCOL CITATION

Andrea Argouarch 2020. Feeding 6wp. **protocols.io**
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COLLECTIONS ⓘ

Dural Cell Isolation and Culturing - Collection

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PARENT PROTOCOLS

Part of collection

[Dural Cell Isolation and Culturing - Collection](#)

STEPS MATERIALS

NAME	CATALOG #	VENDOR
DMEM, high glucose, pyruvate	11995073	Thermo Fisher
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
Fetal Bovine Serum	97068-091	Vwr

Observations

- Record observations
 - Biopsy pieces should look dense and dark; it will not change morphology

- Clean and straight edges on the tissue is best
- Outgrowth will vary between a few days to 2 weeks, depending on biopsy
- After a week, gently change media and then feed every 2-3 days
 - o Be careful not to move coverslip and to not tilt plate

Preparation

- 2 Turn off UV lights and clean hood with 70% ethanol
- 3 Clean items with 70% ethanol and bring into hood
 - a. Sterile Filtered Media with PenStrep



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

Culturing

- 4 Carefully remove old media with vacuum line, making sure not to touch the coverslip.
- 5 Do not tilt plate while feeding to prevent coverslip from sliding
- 6 Add 3 mls  3 mL of new media per well, making sure not to touch the coverslip
 - a. Add media slowly
 - b. Make sure the coverslip is still firmly and evenly over the biopsy
- 7 Gently place 6 well plate in incubator (5% CO₂, 37°C)  37 °C

- 8 Continue to gently feed every 2 – 3 days for 3 weeks to allow enough cells to emerge and expand from tissue

Clean Up

- 9 Throw away biohazard materials properly
- 10 Clean and sterilize hood with 70% ethanol and turn on UV
- 11 Update cell culture notes in lab notebook