

Submission ID #: 70909

Scriptwriter Name: Sri Lakshmi Priya

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Title: Isolation and Culture of Mouse Brain Microvascular Endothelial Cells

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES**

If **Yes**, we will need you to record using screen recording software.

We recommend using the screen capture program [OBS](#). JoVE's tutorial for using OBS Studio is provided at this link: <https://review.jove.com/v/5848/screen-capture-instructions-for-authors?status=a7854k>

As these files are necessary for finalizing your script, **please upload all screen captured video files to your project page as soon as possible.**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **07/15/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 28

Number of Shots: 58

Interviews

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. **Carlos Escudero:** Our study examines how brain endothelial cells can be isolated and cultured to investigate neurovascular function in health and disease.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Carlos Escudero:** The protocol can be used to study fetal brain endothelium in neurodevelopmental and pregnancy-related disorders.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Carlos Escudero:** This approach enables the study of brain endothelial function, angiogenesis, and barrier integrity in vitro.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.5. **Carlos Escudero:** A key challenge in this protocol is careful tissue dissection and meninges removal to prevent contamination with non-endothelial cells.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.6. **Carlos Escudero:** This procedure allows downstream analyses such as gene expression, protein profiling, and functional assays including permeability and angiogenesis

Ethics Title Card

This research has been approved by the Bioethics and Biosafety Committee at the University of Bío-Bío

Protocol

2. Isolation and Preparation of Brain Tissue for Cell Culture

Demonstrator: Esthefanny Escudero-Guevara

- 2.1. To begin, obtain five postnatal day 5 pups [1]. After euthanizing the pups, collect their brains under a laminar-flow biological safety cabinet [2-TXT] and place them in a 50-milliliter conical tube containing sterile, cold PBS (P-B-S) [3].
 - 2.1.1. WIDE: Talent with 5 pups placed on the workbench.
 - 2.1.2. Talent collecting the brains of the pups in a laminar-flow biological safety cabinet. **TXT: Anesthesia: 0.25% Isoflurane followed by decapitation**
 - 2.1.3. Talent placing brains into a conical tube containing PBS.
- 2.2. Roll each brain carefully on a sterilized nitrocellulose membrane to separate the meninges [1]. Transfer the brains to a 100-millimeter plate containing cold PBS [2]. Using a sterile scalpel, remove the brainstem, cerebellum, thalamus, and olfactory bulb [3].
 - 2.2.1. Talent rolling brain tissue on a nitrocellulose membrane.
 - 2.2.2. Talent transferring brains to a plate with PBS.
 - 2.2.3. Talent dissecting and removing brainstem, cerebellum, thalamus, and olfactory bulb with a scalpel.
- 2.3. After collecting the meninges-free cerebral cortices, immediately transfer all the brain cortex to a 50-milliliter conical tube containing 5 milliliters of PBS on ice [1].
 - 2.3.1. Talent transferring cortices into a 50 mL conical tube on ice.
- 2.4. Using sterile scissors, mechanically dissociate the tissue [1]. Using a 1 milliliter pipette, pipette the tissue until a homogeneous suspension without visible fragments is obtained [2].
 - 2.4.1. Talent cutting tissue with scissors.
 - 2.4.2. Talent pipetting repeatedly to homogenize the suspension.
- 2.5. Transfer the sample to a sterile 15-milliliter conical tube containing 2 milliliters of collagenase type II solution [1]. Place the tube in an orbital shaker at 37 degrees Celsius for 2 hours at 180 rpm [2].
 - 2.5.1. Talent adding sample to a tube containing collagenase solution.
 - 2.5.2. Talent placing the tube in an orbital shaker.

- 2.6. Add 5 milliliters of Dulbecco's Modified Eagle Medium or DMEM (*D-M-E-M*) supplemented with 10 percent FBS (*F-B-S*) to the suspension [1]. Centrifuge at 3000 *g* for 10 minutes [2].
 - 2.6.1. Talent adding medium to the digested sample.
 - 2.6.2. Talent placing the tube in a centrifuge.
- 2.7. After discarding the supernatant, resuspend the pellet in 4 milliliters of 20 percent BSA (*B-S-A*) in PBS [1]. Centrifuge the cells at 3000 *g* for 20 minutes [2]. Using a Pasteur pipette, remove the top milky layer containing myelin and discard the remaining supernatant [3].
 - 2.7.1. Talent resuspending pellet in BSA solution.
 - 2.7.2. Talent placing the tube in a centrifuge.
 - 2.7.3. Talent removing the top layer with a Pasteur pipette and discarding the supernatant.
- 2.8. Resuspend the pellet in 2 milliliters of DMEM containing 10 percent FBS [1] and centrifuge again for 10 minutes [2]. After discarding the supernatant, gently resuspend the final pellet in 1 milliliter of DMEM supplemented with 10% FBS [3].
 - 2.8.1. Talent resuspending pellet in medium.
 - 2.8.2. Talent placing the tube in a centrifuge.
 - 2.8.3. Talent resuspending the pellet in medium.
- 2.9. Then, remove the coating solution from the prepared culture plates [1] and rinse each well twice with sterile PBS [2].
 - 2.9.1. Talent removing the coating solution from the culture plates.
 - 2.9.2. Talent adding PBS to the culture plates.
- 2.10. Seed the resuspended cells onto the coated plates and allow the cells to grow until they reach 90-100% confluence [1-TXT].
 - 2.10.1. Talent adding cell suspension to culture dishes. **TXT: Incubation: 37 °C, 5% CO₂**

3. Isolation of Brain Microvascular Endothelial Cells Using CD31 Magnetic Beads

- 3.1. After washing the cultured cells three times with PBS, add 1 milliliter of 0.5 percent trypsin to the plate [1]. Incubate at 37 degrees Celsius for 3 to 5 minutes to detach the cells [2].
 - 3.1.1. Talent adding trypsin to cells.
 - 3.1.2. Talent placing plate into the incubator.
- 3.2. Add 1 milliliter of prepared endothelial culture medium containing 10 percent FBS to neutralize the trypsin [1]. After transferring approximately 2 milliliters of the suspension to a microcentrifuge tube, centrifuge at 220 *g* for 10 minutes at 4 degrees Celsius [2] and discard the supernatant [3].

- 3.2.1. Talent adding medium to cells.
- 3.2.2. Talent placing the microcentrifuge tube into the centrifuge.
- 3.2.3. Talent discarding the supernatant.
- 3.3. Next, add 1 milliliter of 1 percent Hank's solution in a 15-milliliter tube containing 8 microliters of CD31 (*C-D-thirty one*) magnetic beads [1]. After resuspending the cells by swirling the tube, place the tube on a magnet for 2 minutes [2], remove the supernatant, and repeat the wash three times [3].
 - 3.3.1. Talent adding Hank's solution into a tube containing beads.
 - 3.3.2. Talent placing tube on magnet.
 - 3.3.3. Talent removing the supernatant.
- 3.4. Then, resuspend the cell pellet in 1 milliliter of 1 percent Hank's solution containing FBS [1]. Using a 1000 microliter pipette, transfer the cells into the tube containing the prewashed beads [2] and incubate on ice with gentle shaking for 20 minutes [3].
 - 3.4.1. Talent resuspending cells.
 - 3.4.2. Talent transferring the cells into a tube using a pipette.
 - 3.4.3. Talent placing the tube on ice.
- 3.5. Place the tube on a magnet for 3 minutes to precipitate bead-bound endothelial cells [1].
 - 3.5.1. Talent placing tube on magnet.
- 3.6. After removing the supernatant, wash the bead-bound cells with 2 milliliters of Hank's solution three times [1].
 - 3.6.1. Talent adding Hank's solution to the cells.
- 3.7. Resuspend the bead-bound endothelial cells in 7 milliliters of complete endothelial medium containing 10 percent FBS [1].
 - 3.7.1. Talent adding complete endothelial medium to the cells.
- 3.8. Seed the cells onto a 60-millimeter collagen-coated plate [1] and place it in an incubator at 37 degrees Celsius with 5 percent carbon dioxide [2].
 - 3.8.1. Talent adding cells onto a 60 mm collagen coated plate.
 - 3.8.2. Talent placing the plate into an incubator.
- 4. **Characterization of Brain Microvascular Endothelial Cells (BMECs) Using Immunofluorescence**
 - 4.1. Seed 2000 brain microvascular endothelial cells or BMECs (*B-M-E-C-S*) on collagen-coated coverslips [1].
 - 4.1.1. Talent adding cells onto coverslips.

- 4.2. Once the cells attain 80 to 90 percent confluence, add 4 percent paraformaldehyde to fix the cells for 10 minutes at room temperature [1] and wash with PBS [2].
 - 4.2.1. Talent adding paraformaldehyde on the cells.
 - 4.2.2. Talent adding PBS to the cells.
- 4.3. Then, add 0.1 percent Triton X-100 (*X-hundred*) for 5 minutes to permeabilize the cells [1]. Add 5 percent BSA to block non-specific binding for 30 minutes [2].
 - 4.3.1. Talent adding Triton X-100 onto the cells.
 - 4.3.2. Talent adding BSA onto the cells.
- 4.4. Next, add primary antibodies against CD31 and GLUT1 (*Gloot-one*) [1] and incubate overnight at 4 degrees Celsius [2].
 - 4.4.1. Talent adding the antibodies.
 - 4.4.2. Talent placing the samples for incubation in a refrigerator.
- 4.5. After washing the cells with PBS, add fluorescent secondary antibodies and incubate for 1 hour [1].
 - 4.5.1. Talent adding secondary antibodies and plates are being left undisturbed.
- 4.6. Counterstain nuclei with DAPI (*Dapi*) [1] and mount coverslips with antifade medium [2].
 - 4.6.1. Talent adding stain on the cells.
 - 4.6.2. Shot of mounting coverslips with antifade medium.

NOTE: Insert a title card here with the section title *Tube Formation Assay for BMECs Identification*

- 4.7. Precoat a 96-well plate with Matrigel [1]. After allowing it to polymerize for 30 minutes at 37 degrees Celsius, seed 10000 BMECs per well in 100 microliters of complete endothelial medium [2]. Incubate at 37 degrees Celsius to monitor tube formation [3].
 - 4.7.1. Talent placing Matrigel into the wells of a 96-well plate for precoating.
 - 4.7.2. Talent seeding cells into each well containing complete endothelial medium.
 - 4.7.3. Talent placing the plate into an incubator.

NOTE: Insert a title card here with the section title *Measurement of Transendothelial Electrical Resistance*

- 4.8. Seed 20000 BMECs on collagen-coated Transwell inserts with a pore size of 0.4 micrometers in the upper compartment [1].
 - 4.8.1. Talent seeding cells onto Transwell inserts.
- 4.9. Once the cells reach the desired confluence, use an epithelial voltohmmeter to measure transendothelial electrical resistance and assess monolayer integrity [1].

Record the resistance values daily until stabilization [2] and confirm the formation of tight intercellular junctions through an increase in resistance [3].

4.9.1. Talent measuring resistance using a voltohmmeter.

4.9.2. SCREEN: 70909_screenshoot_1.mp4: 00:03-00:09, 00:35-00:40

4.9.3. SCREEN: 70909_screenshoot_2.mp4: 00:06-00:12

4.10. Seed the non-selected cell mixture in the bottom well of the culture plate [1] and BMECs on the upper side of the Transwell insert membrane [2].

4.10.1. Talent adding non-selected cells into the lower compartment.

4.10.2. Talent adding endothelial cells onto the upper insert membrane.

4.11. After expansion and maintenance of purified BMECs, store the cells in liquid nitrogen [1].

4.11.1. Talent placing the plates into a liquid nitrogen storage unit.

Results

5. Results

- 5.1. At day 5 post-isolation, the total cell pool consisted of a mixed cell population prior to immunoselection [1].
 - 5.1.1. LAB MEDIA: Figure 2A.
- 5.2. Following CD31-based magnetic bead selection, endothelial cells were enriched, with bead-associated cells observed after selection [1].
 - 5.2.1. LAB MEDIA: Figure 2B.
- 5.3. BMECs showed progressive expansion between days 3 and 7 after immunoselection [1] and formed confluent monolayers with a cobblestone morphology [2].
 - 5.3.1. LAB MEDIA: Figure 2C–E. *Video editor: Show the full panel labeled C, D, and E.*
 - 5.3.2. LAB MEDIA: Figure 2C–E. *Video editor: Emphasize the figure 2E.*
- 5.4. CD31 positive staining was observed at day 7 post-isolation, confirming endothelial identity [1].
 - 5.4.1. LAB MEDIA: Figure 3A. *Video editor: Emphasize the image labeled CD31 and MERGE.*
- 5.5. GLUT1 expression confirmed the brain endothelial phenotype [1].
 - 5.5.1. LAB MEDIA: Figure 3B. *Video editor: Emphasize the image labeled GLUT-1 and MERGE.*
- 5.6. Glial fibrillary acidic protein and ionized calcium-binding adaptor molecule 1 staining showed no detectable signal [1], indicating the absence of astrocytes and microglia [2].
 - 5.6.1. LAB MEDIA: Figure 3C, D. *Video editor: Emphasize the image under GFAP and IBA1.*
 - 5.6.2. LAB MEDIA: Figure 3C, D.
- 5.7. In vitro tube formation assays showed the formation of capillary-like tubular structures within 6 hours of seeding [1].
 - 5.7.1. LAB MEDIA: Figure 4A.
- 5.8. Quantitative analysis of angiogenesis parameters, including total tube length [1], number of junctions [2], and number of branches, was performed [3].

- 5.8.1. LAB MEDIA: Figure 4B. *Video editor: Emphasize the light blue line labeled as meshes.*
- 5.8.2. LAB MEDIA: Figure 4B. *Video editor: Emphasize the red blue circles with red dots in center labelled as junctions.*
- 5.8.3. LAB MEDIA: Figure 4B. *Video editor: Emphasize the green line with yellow dots labeled as branches.*

- 5.9. Higher TEER values were observed in coculture conditions [1] compared to monoculture [2].
 - 5.9.1. LAB MEDIA: Figure 4C. *Video editor: Emphasize the red line labelled as coculture.*
 - 5.9.2. LAB MEDIA: Figure 4C. *Video editor: Emphasize the blue line labelled as monoculture.*

- 1. Isoflurane
 Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
 IPA: /ˌaɪsoʊˈfloʊrɪn/
 Phonetic Spelling: eye·soh·FLOOR·ayn
- 2. Decapitation
 Pronunciation link: <https://www.merriam-webster.com/dictionary/decapitation>
 IPA: /dɪˌkæpəˈteɪʃən/
 Phonetic Spelling: dih·kap·uh·TAY·shuhn
- 3. Nitrocellulose
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nitrocellulose>
 IPA: /ˌnaɪtroʊˈseljəˌloʊs/
 Phonetic Spelling: nye·troh·SEL·yuh·lohs
- 4. Meninges
 Pronunciation link: <https://www.merriam-webster.com/dictionary/meninges>
 IPA: /məˈnɪndʒiːz/
 Phonetic Spelling: muh·NIN·jeez
- 5. Brainstem
 Pronunciation link: <https://www.merriam-webster.com/dictionary/brainstem>
 IPA: /ˈbreɪnˌstɛm/
 Phonetic Spelling: BRAYN·stem
- 6. Cerebellum
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebellum>
 IPA: /ˌsɛrəˈbɛləm/
 Phonetic Spelling: sair·uh·BEL·uhm
- 7. Thalamus
 Pronunciation link: <https://www.merriam-webster.com/dictionary/thalamus>
 IPA: /ˈθæləməs/
 Phonetic Spelling: THAL·uh·muhs

8. Olfactory
 Pronunciation link: <https://www.merriam-webster.com/dictionary/olfactory>
 IPA: /ɑːlˈfæktəri/
 Phonetic Spelling: ol·FAK·tuh·ree
9. Cerebral
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral>
 IPA: /səˈriːbrəl/; /ˈserəbrəl/
 Phonetic Spelling: suh·REE·bruhl; SAIR·uh·bruhl
10. Cortices
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cortex>
 IPA: /ˈkɔːrtɪˌsiːz/
 Phonetic Spelling: KOR·tih·seez
11. Collagenase
 Pronunciation link: <https://www.merriam-webster.com/medical/collagenase>
 IPA: /kəˈlædʒəˌneɪs/
 Phonetic Spelling: kuh·LAJ·uh·nays
12. Dulbecco's
 Pronunciation link: No confirmed link found
 IPA: /dʌlˈbɛkoʊz/
 Phonetic Spelling: dul·BEK·ohz
13. Centrifuge
 Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
 IPA: /ˈsentrəˌfjuːdʒ/
 Phonetic Spelling: SEN·truh·fyooj
14. Supernatant
 Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
 IPA: /ˌsuːpərˈneɪtənt/
 Phonetic Spelling: soo·per·NAY·tuht
15. Pasteur
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Pasteur>
 IPA: /pæˈstɜːr/
 Phonetic Spelling: pa·STUR
16. Myelin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/myelin>
 IPA: /ˈmaɪəlɪn/
 Phonetic Spelling: MY·uh·lin
17. Confluence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
 IPA: /ˈkɑːnfluəns/
 Phonetic Spelling: KON·floo·uhns
18. Microvascular
 Pronunciation link: No confirmed link found
 IPA: /ˌmaɪkroʊˈvæskjələr/
 Phonetic Spelling: my·kroh·VAS·kyuh·ler

19. Endothelial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/endothelial>
 IPA: /,ɛndəʊˈθiːliəl/
 Phonetic Spelling: en·doh·THEE·lee·uhl
20. Trypsin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
 IPA: /ˈtrɪpsɪn/
 Phonetic Spelling: TRIP·sin
21. Microcentrifuge
 Pronunciation link: No confirmed link found
 IPA: /,maɪkroʊˈsentrəˌfjuːdʒ/
 Phonetic Spelling: my·kroh·SEN·truh·fyooj
22. Immunofluorescence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/immunofluorescence>
 IPA: /ɪˌmjuːnoʊflɒˈrɛsəns/
 Phonetic Spelling: ih·MYOO·noh·floor·ES·uhns
23. Paraformaldehyde
 Pronunciation link: No confirmed link found
 IPA: /,pærəfɔːrˈmældəˌhaɪd/
 Phonetic Spelling: pair·uh·for·MAL·duh·hyde
24. Permeabilize
 Pronunciation link: No confirmed link found
 IPA: /ˈpɜːrmɪəˌbaɪəz/
 Phonetic Spelling: PER·mee·uh·bih·lyze
25. Antibodies
 Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>
 IPA: /ˈæntɪˌbɑːdɪz/
 Phonetic Spelling: AN·tee·bod·eez
26. Fluorescent
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>
 IPA: /flɒˈrɛsənt/
 Phonetic Spelling: floor·ES·uhnt
27. Counterstain
 Pronunciation link: No confirmed link found
 IPA: /ˈkaʊntərˌstem/
 Phonetic Spelling: KOWN·ter·stayn
28. DAPI
 Pronunciation link: No confirmed link found
 IPA: /ˈdæpi/
 Phonetic Spelling: DAP·ee
29. Antifade
 Pronunciation link: No confirmed link found
 IPA: /ˌæntɪˈfeɪd/
 Phonetic Spelling: an·tee·FAYD

30. Matrigel
 Pronunciation link: No confirmed link found
 IPA: /'mætrɪ,dʒəl/
 Phonetic Spelling: MAT·rih·jel
31. Polymerize
 Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerize>
 IPA: /pə'limə,raɪz/
 Phonetic Spelling: puh·LIM·uh·ryze
32. Transendothelial
 Pronunciation link: No confirmed link found
 IPA: /,trænz,ɛndoo'thi:liəl/
 Phonetic Spelling: tranz·en·doh·THEE·lee·uhl
33. Voltohmmeter
 Pronunciation link: No confirmed link found
 IPA: /voolt'oomitər/
 Phonetic Spelling: vohlt·OH·mih·ter
34. Monolayer
 Pronunciation link: <https://www.merriam-webster.com/dictionary/monolayer>
 IPA: /'mɑ:noo,leɪər/
 Phonetic Spelling: MON·oh·lay·er
35. Intercellular
 Pronunciation link: <https://www.merriam-webster.com/dictionary/intercellular>
 IPA: /,ɪntər'seljələr/
 Phonetic Spelling: in·ter·SEL·yuh·ler
36. Immunoselection
 Pronunciation link: No confirmed link found
 IPA: /ɪ,mju:nousə'lekʃən/
 Phonetic Spelling: ih·MYOO·noh·suh·LEK·shuhn
37. Cobblestone
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cobblestone>
 IPA: /'kɑ:bəl,stoʊn/
 Phonetic Spelling: KOB·uhl·stohn
38. Phenotype
 Pronunciation link: <https://www.merriam-webster.com/dictionary/phenotype>
 IPA: /'fi:nə,taɪp/
 Phonetic Spelling: FEE·nuh·type
39. Glial
 Pronunciation link: <https://www.merriam-webster.com/dictionary/glia>
 IPA: /'glaiəl/
 Phonetic Spelling: GLY·uhl
40. Fibrillary
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fibrillary>
 IPA: /'faɪbrə,leri/
 Phonetic Spelling: FY·bruh·lair·ee

41. Astrocytes
Pronunciation link: <https://www.merriam-webster.com/dictionary/astrocyte>
IPA: /'æstrəˌsaɪts/
Phonetic Spelling: AS·truh·sytes
42. Microglia
Pronunciation link: <https://www.merriam-webster.com/dictionary/microglia>
IPA: /maɪˈkraːɡliə/
Phonetic Spelling: my·KROG·lee·uh
43. Angiogenesis
Pronunciation link: <https://www.merriam-webster.com/dictionary/angiogenesis>
IPA: /ˌændʒiəʊˈdʒenəsis/
Phonetic Spelling: an·jee·oh·JEN·uh·sis
44. Coculture
Pronunciation link: No confirmed link found
IPA: /ˌkoʊˈkʌltʃər/
Phonetic Spelling: koh·KUL·cher
45. Monoculture
Pronunciation link: <https://www.merriam-webster.com/dictionary/monoculture>
IPA: /ˈməːnoʊˌkʌltʃər/
Phonetic Spelling: MON·oh·kul·cher