

Submission ID #: 69749

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=21273493>

## **Title: Isolation of Pericytes from Mouse Cortical Tissue Using FACS for Single-Cell Sequencing**

### **Authors and Affiliations:**

Xinyi Cui<sup>1,2,\*</sup>, Qingbin Wu<sup>1,2,\*</sup>, Shuang Zhang<sup>1,2</sup>, Xueting Liu<sup>1,2</sup>, Hongwei Li<sup>1,2</sup>,  
Xiaochen Yuan<sup>1,2</sup>, Anping Xu<sup>3</sup>, Ruijuan Xiu<sup>1,2</sup>

<sup>1</sup>Institute of Microcirculation, Chinese Academy of Medical Sciences & Peking Union Medical College

<sup>2</sup>International Center of Microvascular Medicine, Chinese Academy of Medical Sciences

<sup>3</sup>College of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine

**\*These authors contributed equally**

### **Corresponding Authors:**

Xiaochen Yuan

[yuanxc@imc.pumc.edu.cn](mailto:yuanxc@imc.pumc.edu.cn)

Anping Xu

[700558@bucm.edu.cn](mailto:700558@bucm.edu.cn)

### **Email Addresses for All Authors:**

Xinyi Cui

[cui28494694@163.com](mailto:cui28494694@163.com)

Qingbin Wu

[519706@163.com](mailto:519706@163.com)

Shuang Zhang

[zhangshuang@imc.pumc.edu.cn](mailto:zhangshuang@imc.pumc.edu.cn)

Xueting Liu

[18519231305@163.com](mailto:18519231305@163.com)

Hongwei Li

[lihw66@imc.pumc.edu.cn](mailto:lihw66@imc.pumc.edu.cn)

Xiaochen Yuan

[yuanxc@imc.pumc.edu.cn](mailto:yuanxc@imc.pumc.edu.cn)

Anping Xu

[700558@bucm.edu.cn](mailto:700558@bucm.edu.cn)

Ruijuan Xiu

[xiurj@imc.pumc.edu.cn](mailto:xiurj@imc.pumc.edu.cn)

## **Author Questionnaire**

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar?

**Yes, all done**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

### **Current Protocol Length**

Number of Steps: 30

Number of Shots: 58

# Interviews

---

*Videographer: Obtain headshots for all authors available at the filming location.*

## INTRODUCTION:

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

- 1.1. **Xinyi Cui:** Our research examines brain pericytes, focusing on their vascular roles and interactions within the neurovascular unit to understand cerebral function.

- 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~What technologies are currently used to advance research in your field?~~

- 1.2. **Xinyi Cui:** Live imaging and spatial omics enable precise mapping of pericyte function within brain vasculature, revealing location-specific roles and therapeutic targets.

- 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

## CONCLUSION:

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

- 1.5. **Qingbin Wu:** This protocol allows researchers to isolate high-purity, viable pericytes suitable for downstream single-cell sequencing and functional analyses.

- 1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.6. **Xinyi Cui:** The main challenge is the absence of highly specific pericyte markers, complicating pure isolation and increasing the risk of cellular contamination.

- 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.7. **Qingbin Wu:** Future studies can focus on identifying pericyte-specific markers, enabling improved isolation, deeper functional characterization, and targeted therapeutic investigations.

1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

***Videographer: Obtain headshots for all authors available at the filming location.***

**Ethics Title Card**

This research has been approved by the Laboratory Animal Care and Ethics Committee of the Institute of Microcirculation, Chinese Academy of Medical Sciences, and Peking Union Medical College

# Protocol

NOTE: SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

## 2. Mouse Brain Dissection and Cerebral Cortex Isolation

**Demonstrator:** Xinyi Cui

- 2.1. To begin, spray the head and neck region of a euthanized mouse with 75 percent ethanol [1-TXT]. Using a scalpel, make a midline scalp incision from the occipital to the frontal region to expose the skull [2].
  - 2.1.1. WIDE: Talent spraying the head and neck region with ethanol. **TXT: Anesthesia: Tribromoethanol (0.2 mL/10 g body weight) (i.p) followed by cervical dislocation**
  - 2.1.2. Talent making a midline scalp incision to expose the skull.
- 2.2. Perform a transverse incision anterior to the bilateral orbits [1], followed by lateral incisions along the inferior margins of the cerebellum to detach the skull base [2].
  - 2.2.1. Talent performing a transverse incision anterior to both orbits.
  - 2.2.2. Talent making lateral incisions along the inferior margins of the cerebellum.
- 2.3. Incise the calvarium longitudinally along the sagittal suture from posterior to anterior [1]. Lift the cranial flap together with the dura mater to expose the brain [2].
  - 2.3.1. Talent cutting along the sagittal suture of the skull.
  - 2.3.2. Talent lifting the cranial flap to reveal the brain.
- 2.4. Using surgical scissors, gently elevate the brain while severing the optic nerves and the medulla oblongata–spinal cord connection [1].
  - 2.4.1. Talent cutting the optic nerves and brainstem connection with scissors.
- 2.5. Place the intact brain gently into a 10-centimeter dish containing 10 milliliters of ice-cold PBS (P-B-S), avoiding any damage [1]. ~~Handle the tissue gently to avoid damage [2].~~ NOTE: For removed shot, VO is added in concise format in previous step
  - 2.5.1. Talent placing the intact brain into a dish with cold buffer.
  - 2.5.2. ~~Talent handling the tissue gently.~~ NOTE: Author removed the shot and added the VO concisely with previous step.
- 2.6. Transfer the brain onto aluminum foil [1]. ~~Using forceps, lift the dura mater from the margins and excise it along the midline sulci [2]. After complete dura removal, expose the subarachnoid space [3].~~

- 2.6.1. Talent placing the brain on aluminum foil.
- 2.6.2. ~~SCOPE: Talent removing the dura mater with forceps.~~ **Author's NOTE: Remove it**
- 2.6.3. SCOPE: JOVE\_LM\_69749\_Step2.6.2\_Shot-showing-the-exposed-subarachnoid-space..mp4: 00:07-00:19
  
- 2.7. Rinse the subarachnoid space with Hanks balanced salt solution [1]. Transfer the tissue to filter paper and remove the pia mater by rolling [2].
  - 2.7.1. SCOPE:JOVE\_LM\_69749\_Step2.7.1\_Talent-pipetting-HBSS-buffer-over-the-subarachnoid-space..mp4: 00:00-00:27
  - 2.7.2. SCOPE:JOVE\_LM\_69749\_Step2.7.2\_Talent-transferring-the-tissue-to-filter-paper-and-rolling-it-to-remove-the-pia-mater..mp4: 00:03-00:32
  
- 2.8. With a razor blade, cut and discard the olfactory bulb and the caudal-most sections [1] and section the brain coronally into slices 2 to 3 millimeters thick [2]. **NOTE: VO is also edited for inverted shots.**
  - 2.8.2. SCOPE:JOVE\_LM\_69749\_Step2.8.1\_Talent-cutting-the-brain-into-coronal-sections-and-discarding-unwanted-sections..mp4: 00:00-01:24
  - 2.8.1 SCOPE:JOVE\_LM\_69749\_Step2.8.1\_Talent-cutting-the-brain-into-coronal-sections-and-discarding-unwanted-sections..mp4: 00:00-01:24

**NOTE: 2.8.2 was placed before 2.8.1 as per the scope video. Show the scope shot only once.**

**Author's NOTE: 2.8.1 and 2.8.2 are combined in one shot.**
  
- 2.9. Isolate the cerebral cortex along the corpus callosum [1]. Transfer the cortical tissue into ampoules containing 3 milliliters of ice-cold tissue collection buffer [2].
  - 2.9.1. SCOPE: JOVE\_LM\_69749\_Step2.9.1\_Talent-isolating-the-cerebral-cortex..mp4: 00:13-00:42, 01:18-01:52
  - 2.9.2. Talent transferring cortical tissue into ampoules with buffer.

### **3. Tissue Dissociation and Density Gradient–Based Cell Enrichment**

- 3.1. Using surgical scalpels, mince the tissue in ampoules into fragments of approximately 1 by 1 millimeter and complete the mincing within 5 minutes to preserve tissue freshness [1].
  - 3.1.1. Talent mincing tissue with scalpels.
- 3.2. Pre-rinse pipette tips with tissue collection buffer [1]. Then slowly aspirate the minced fragments toward the tip apex while holding the pipette vertically [2]. Gently expel the fragments into pre-chilled 15-milliliter centrifuge tubes [3].

- 3.2.1. Talent rinsing pipette tips with buffer.
- 3.2.2. Talent aspirating tissue fragments vertically.
- 3.2.3. Talent dispensing fragments into centrifuge tubes.
- 3.3. Wash the original ampoules with 2 percent FBS (*F-B-S*) in PBS saline [1]. Transfer the wash into the same centrifuge tubes and centrifuge at 300 g for 5 minutes at 4 degrees Celsius [2].
  - 3.3.1. Talent rinsing ampoules with 2% FBS in PBS.
  - 3.3.2. Talent transferring the wash into centrifuge tubes.
- 3.4. Discard the supernatant after centrifugation [1]. Resuspend each pellet in 3 milliliters of CD (*C-D*) enzyme working solution [2]. Aliquot the suspension equally into three tubes and add additional enzyme solution to reach a final volume of 5 milliliters per tube [3].
  - 3.4.1. Talent aspirating and discarding supernatants.
  - 3.4.2. Talent resuspending pellets in enzyme solution.
  - 3.4.3. Talent aliquoting the suspension into 3 tubes and adjusting final volume to 5 ml with enzyme solution.
- 3.5. Incubate the tubes in a hybridization oven at 37 degrees Celsius for 100 minutes with orbital shaking at 100 g [1].
  - 3.5.1. Talent placing tubes into the oven.
- 3.6. During incubation, add 1 milliliter of DNase I (*D-N-Ase-One*) solution to 10 milliliters of 2 percent FBS in PBS [1]. Terminate digestion centrifuging at 300 g at 4 degrees Celsius for 5 minutes [2].
  - 3.6.1. Talent preparing the dissociation solution at the bench.
  - 3.6.2. Talent loading tubes into the centrifuge.
- 3.7. Then resuspend each pellet in 1 milliliter of tissue dissociation solution [1]. Pipette the solution 100 times to aid mechanical dissociation [2].
  - 3.7.1. Talent resuspending the pellet in 1 mL of tissue dissociation solution.
  - 3.7.2. Talent repeatedly pipetting to dissociate tissue.
- 3.8. Next, pool all suspensions into a single 50-milliliter centrifuge tube [1]. Rinse each tube with 2 milliliters of buffer and combine the washes [2].
  - 3.8.1. Talent pooling cell suspensions.
  - 3.8.2. Talent rinsing and combining washes.
- 3.9. After centrifugation at 300g for 5 minutes at 4 degrees Celsius and discarding the uppermost layer, add 12.5 milliliters of 20 percent BSA (*B-S-A*) and invert to mix [1].

Centrifuge at 1,000 *g* at 4 degrees Celsius for 20 minutes [2].

3.9.1. Talent adding bovine serum albumin solution and inverting the tubes

3.9.2. Talent placing the tube into the centrifuge.

3.10. Now collect the bottom pellet and transfer the supernatant into a new 50 milliliter centrifuge tube [1]. Resuspend gently before centrifuging again [2].

3.10.1. Talent separating pellet and supernatant.

3.10.2. Talent loading tubes for the second centrifugation.

3.11. Resuspend each pellet in 2 milliliters of 2 percent FBS in PBS [1]. Then combine both suspensions into a single tube [2].

3.11.1. Talent resuspending pellets in 2 mL of FBS in PBS.

3.11.2. Talent combining the suspensions.

3.12. Centrifuge the combined suspension at 300 *g* at 4 degrees Celsius for 5 minutes [1]. After discarding the supernatant, resuspend the final pellet in 2 milliliters of PBS with 2% FBS [2].

3.12.1. Talent placing the combined suspension in a centrifuge.

3.12.2. Talent resuspending the final purified cells in 2 mL PBS.

3.13. Prepare four 15-milliliter centrifuge tubes containing 4 milliliters of 22 percent Percoll solution [1]. Gently layer 0.5 milliliter aliquots of the cell suspension onto each gradient and centrifuge at 560*g* for 10 minutes at 4 degrees Celsius [2].

3.13.1. Talent preparing Percoll gradients.

3.13.2. Talent layering cell suspensions onto the gradients.

3.14. After aspirating the supernatant, resuspend each pellet in 1 milliliter of HBSS (H-B-S-S) BSA glucose buffer [1]. Then pool the suspensions into a new 15-milliliter tube [2]. Rinse the Percoll tubes with 2 milliliters of buffer and combine the washes before centrifugation at 300 *g* for 5 minutes at 4 degrees Celsius [3].

3.14.1. Talent resuspending Percoll pellets in 1 mL HBSS/BSA/Glucose buffer.

3.14.2. Shot of the suspensions being pooled into a 15 mL tube.

3.14.3. Shot of the washes being combined into the 15 mL tube.

#### **4. Flow Cytometry Staining and Preparation of Brain-Derived Cells for Sorting**

4.1. For flow cytometry, resuspend the pellet in 1 milliliter of HBSS BSA glucose buffer after aspirating the supernatant [1].

4.1.1. Talent resuspending the cell pellet in 1 mL of HBSS/BSA/glucose buffer.

4.2. Add 5 microliters of Fc (F-C) receptor blocking reagent and incubate at 4 degrees Celsius for 5 minutes [1]. Then, pipette fluorescently conjugated antibodies to the suspension before incubating [2].

4.2.1. Talent adding Fc receptor blocking reagent and placing the tube on ice.

4.2.2. Talent adding antibody reagents to the tube.

AND

**TEXT ON PLAIN BACKGROUND:**

Fluorescently conjugated antibodies:

5  $\mu$ L of CD45-PE

5  $\mu$ L of CD41-PE

20  $\mu$ L of CD31-APC

15  $\mu$ L of CD13-FITC

Incubation: On ice, 20 min in the dark

*Video Editor: Please play both shots side by side in a split screen*

4.3. Wash the cells with 6 milliliters of HBSS BSA glucose buffer [1]. Then centrifuge the suspension at 300 g at 4 degrees Celsius for 5 minutes [2].

4.3.1. Talent adding 6 mL wash buffer to the cells

4.3.2. Talent placing the stained cells in a centrifuge.

4.4. After aspirating the supernatant, resuspend the pellet in 1 milliliter of buffer [1]. Add 200 microliters of 7-aminoactinomycin D solution [2] and incubate on ice for 10 minutes [3]. Then, adjust the final volume to 2 milliliters with HBSS buffer supplemented with BSA and glucose [4]. **NOTE: VO is added for additional shot**

4.4.1. Talent resuspending the pellet in 1mL buffer.

4.4.2. Talent adding 200  $\mu$ L of AAD solution.

4.4.3. Talent incubating the tube on ice.

**Added shot: Talent adding buffer to bring the final volume to 2 mL**

4.5. Rinse the inner surface of sorting collection tubes with 1 percent BSA solution [1]. Aspirate out the excess solution, leaving exactly 1 milliliter of solution in each tube [2]. Store the tubes on ice to receive sorted cells [3]. **NOTE: VO has been edited to match the revised shot.**

4.5.1. ~~Talent coating sorting tubes with bovine serum albumin solution.~~ Talent swirling the sorting collection tubes containing 1% BSA solution

4.5.2. Shot of the excess solution being pipetted out.

4.5.3. Shot of prepared collection tubes resting on ice.



# Results

---

## 5. Results

- 5.1. Cells dissociated from brain tissue were sequentially gated by forward scatter area and side scatter area to exclude cellular debris during flow cytometry sorting [1]. CD45 (*C-D-Forty-five*) -positive and CD41(*C-D-Forty-one*) -positive dual-positive events were excluded to remove hematopoietic cells and platelet contaminants [2].
  - 5.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the top-left plot (SSC-A) and focus on the boxed region*
  - 5.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the top-right plot.*
- 5.2. CD13-positive vascular wall cells were enriched, and apoptotic cells were excluded using 7-aminoactinomycin D negative gating, followed by CD31-negative selection to isolate pericytes [1].
  - 5.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the gated region in the bottom-left plot.*
- 5.3. Flow cytometry sorting showed that nucleated cells accounted for 59.8 percent of all events [1], CD13-positive cells accounted for 1 percent of all events [2], and the final CD13-positive CD31-negative pericyte population accounted for 0.7 percent of all events [3].
  - 5.3.1. LAB MEDIA: Figure 2. *Video editor: Highlight the table entry showing 59.8 percent for nucleated cells.*
  - 5.3.2. LAB MEDIA: Figure 2. *Video editor: Highlight the table entry showing 1.0 percent for the CD13-positive population.*
  - 5.3.3. LAB MEDIA: Figure 2. *Video editor: Highlight the table entry showing 0.7 percent for the CD13-positive CD31-negative population.*
- 5.4. UMAP (*You-Map*) dimensionality reduction of the sorted cells revealed six distinct major clusters, indicating cellular heterogeneity within the isolated population [1].
  - 5.4.1. LAB MEDIA: Figure 3A. *Video editor: Show the full UMAP plot with the six color-coded clusters.*
- 5.5. Z-score normalized heatmap analysis demonstrated that pericyte marker genes were highly and predominantly expressed in clusters 0 through 3 [1].
  - 5.5.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the red boxes in the rows corresponding to clusters 0 to 3*

5.6. Cluster proportion analysis showed that clusters 0 through 3 constituted 87.1 percent of the total population [1], whereas smooth muscle cell markers were enriched in cluster 4 at 10.14 percent [2] and fibroblast markers were enriched in cluster 5 at 2.77 percent [3].

5.6.1. LAB MEDIA: Figure 3C. *Video editor: please highlight the bars corresponding to Clusters 0 to 3*

5.6.2. LAB MEDIA: Figure 3C. *Video editor: please highlight the bars corresponding to Clusters 4*

5.6.3. LAB MEDIA: Figure 3C. *Video editor: please highlight the bars corresponding to Clusters 5*

#### **Pronunciation guides:**

1. Anesthetized  
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>  
IPA: /əˈnesθəˌtaɪzd/  
Phonetic Spelling: uh·ness·thuh·tyzd
2. Occipital  
Pronunciation link: <https://www.merriam-webster.com/dictionary/occipital>  
IPA: /ɑkˈsɪpətəl/  
Phonetic Spelling: ok·sip·uh·tuhl
3. Ethanol  
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>  
IPA: /ˈeθəˌnɒl/  
Phonetic Spelling: eth·uh·nawl
4. Scalpel  
Pronunciation link: <https://www.merriam-webster.com/dictionary/scalpel>  
IPA: /ˈskalpəl/  
Phonetic Spelling: skal·puhl
5. Cerebellum  
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebellum>  
IPA: /ˌserəˈbeləm/  
Phonetic Spelling: seh·ruh·bel·uhm
6. Calvarium  
Pronunciation link: <https://www.merriam-webster.com/dictionary/calvarium>  
IPA: /kalˈveriəm/  
Phonetic Spelling: kal·vair·ee·uhm

7. Sagittal  
Pronunciation link: <https://www.merriam-webster.com/dictionary/sagittal>  
IPA: /'sædʒɪtəl/  
Phonetic Spelling: saj·uh·tuhl
8. Dura Mater  
Pronunciation link: <https://www.merriam-webster.com/dictionary/dura%20mater>  
IPA: /,dʊrə 'mætər/  
Phonetic Spelling: dur·uh maa·ter
9. Optic  
Pronunciation link: <https://www.merriam-webster.com/dictionary/optic>  
IPA: /'ɒptɪk/  
Phonetic Spelling: op·tik
10. Medulla Oblongata  
Pronunciation link: <https://www.merriam-webster.com/dictionary/medulla%20oblongata>  
IPA: /məˌdʌlə ˌɒblŋˈɡatə/  
Phonetic Spelling: muh·dul·uh ob·long·gaa·tuh
11. Subarachnoid  
Pronunciation link: <https://www.merriam-webster.com/dictionary/subarachnoid>  
IPA: /ˌsʌbəˈrækˌnɔɪd/  
Phonetic Spelling: sub·uh·rak·noyd
12. Hanks  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Hanks>  
IPA: /'hæŋks/  
Phonetic Spelling: hanks
13. Pia Mater  
Pronunciation link: <https://www.merriam-webster.com/dictionary/pia%20mater>  
IPA: /ˌpaɪə 'mætər/  
Phonetic Spelling: pie·uh maa·ter
14. Olfactory  
Pronunciation link: <https://www.merriam-webster.com/dictionary/olfactory>  
IPA: /ɒl'fækˌtɔːri/, /oʊl'fækˌtɔːri/  
Phonetic Spelling: ol·fak·tor·ee
15. Coronally  
Pronunciation link: <https://www.merriam-webster.com/dictionary/coronal>  
IPA: /kə'roʊnəl/  
Phonetic Spelling: kuh·roh·nuhl·ee
16. Cerebral Cortex  
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral%20cortex>  
IPA: /səˈriːbrəl 'kɔːteks/  
Phonetic Spelling: suh·ree·bruhl kor·teks
17. Corpus Callosum  
Pronunciation link: <https://www.merriam-webster.com/dictionary/corpus%20callosum>

IPA: /ˌkɔrpəs kəˈloʊsəm/

Phonetic Spelling: kor·pus kuh·loh·suhm

18. Ampoules

Pronunciation link: <https://www.merriam-webster.com/dictionary/ampoule>

IPA: /ˈæmˌpuːl/

Phonetic Spelling: am·pool

19. Dissociation

Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>

IPA: /dɪˌsoʊsiˈeɪʃən/

Phonetic Spelling: dih·soh·see·ay·shuhn

20. Aliquot

Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>

IPA: /ˈælɪkwət/

Phonetic Spelling: al·ih·kwot

21. Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /ˈsentərəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

22. Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /ˌsuːpərˈnextənt/

Phonetic Spelling: soo·per·nay·tuht

23. Resuspend

Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>

IPA: /ˌriːsəˈspend/

Phonetic Spelling: ree·suh·spend

24. Pellet

Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>

IPA: /ˈpelət/

Phonetic Spelling: pel·uht

25. Enzyme

Pronunciation link: <https://www.merriam-webster.com/dictionary/enzyme>

IPA: /ˈenzaim/

Phonetic Spelling: en·zym

26. Tribromoethanol

Pronunciation link: No confirmed link found

IPA: /traɪˌbroʊmoʊˈeθəˌnɔl/

Phonetic Spelling: try·broh·moh·eth·uh·nawl

27. Coronal

Pronunciation link: <https://www.merriam-webster.com/dictionary/coronal>

IPA: /kəˈroʊnəl/

Phonetic Spelling: kuh·roh·nuhl