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**Scriptwriter Name: Pallavi Sharma**

**Project Page Link: [https://review.jove.com/files\\_upload.php?src=22217468](https://review.jove.com/files_upload.php?src=22217468)**

**Title: Dissociation of Murine Mammary Gland and Tumor Tissues to Create Highly Viable Single-Cell Suspensions**

**Authors and Affiliations:**

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| Wei-Zen Wei    | (weiw@karmanos.org) |
| Jennifer Jacob | (wellsje2@msu.edu)  |

## **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? **NO**
  
- 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: 31

Number of Shots: 55

# Interviews

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

**NOTE: Interview lav audio was wired directly to the camera.**

## INTRODUCTION:

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

- 1.1. **Jennifer Jacob:** Our overarching goal is to identify oncogene regulatory genes and use this information to disrupt tumor onset and progression or enhance anti-tumor immunity.
  - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.2. **Jennifer Jacob/Ryann Ray:** This protocol is most useful for collecting single-cell suspensions from murine adipose-rich mammary glands and tumors for downstream applications such as flow cytometry and single-cell sequencing.
  - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

## CONCLUSION:

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

- 1.3. **Ryann Ray:** The most important consideration when performing this protocol is to finely mince the tissue before enzymatic digestion to maximize cell yield and preserve representative cell populations from the original tissue.
  - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.2.2*

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

- 1.4. **Jennifer Jacob** : Following this procedure, specific cell subsets can be isolated for ex vivo immune cell functional assays, in addition to protein and RNA expression analyses.

1.4.1. 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.~~

- 1.5. **Jennifer Jacob**: Future research will investigate how this basic protocol can be modified for application in other organs.

1.5.1. 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.***

### **Promotional Interview Questions:**

***Videographer: Please capture these shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Statements will be presented live by the authors, sharing their spontaneous perspectives.***

You mention the whole workflow takes about three hours. What does a typical day in the lab running this look like?

- 1.6. **Enter author name**: (authors will present their testimonial statements live)  
1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. **NOTE: The name of the person who delivered the statement is not provided**

Mammary fat pad is notoriously difficult to work with. What made you look for a better cell isolation protocol?

- 1.7. **Enter author name**: (authors will present their testimonial statements live)  
1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University.

# Protocol

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***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

**Videographer's NOTE:** Some shots were combined into one continuous take, for these shots we verbally called out the individual shots throughout the take. The authors had pre-existing headshots and opted to provide those to JoVE. All footage has been uploaded via AWS FTP. There are 46 files total. Sub Folder Name: 72844\_Aug\_5\_2026\_JenniferJacob

## 2. Harvesting Mammary Tumors or Fat Pads for Tissue Digestion

**Demonstrator:** Ryann Ray

- 2.1. After exposing the body cavity of the euthanized mouse [1], remove the medium-sized tumors and the mammary glands or fat pads [1]. Place the tissue in a 50-milliliter conical tube containing 5 milliliters of R10 medium supplemented with 10 percent FBS, excluding necrotic or blood-filled tumors whenever possible [2].
  - 2.1.1. Establishing shot of the talent with the prepared mouse placed in front.
  - 2.1.2. Talent removing the medium-sized tumor and the mammary gland .
  - 2.1.3. Talent placing the tissue into a 50-milliliter conical tube containing 5 milliliters of RPMI medium with 10 percent FBS.
- 2.2. In a biological safety cabinet, transfer only the mammary fat pads and tumors to a clean Petri dish [1]. Using two surgical scalpel blades, mince the tissue until it forms a paste-like consistency [2], then transfer the minced tissue to a clean 50-milliliter tube at room temperature [3].
  - 2.2.1. Talent transferring the mammary fat pads and tumors to a clean Petri dish inside a biological safety cabinet.
  - 2.2.2. Talent mincing the tissue with two surgical scalpel blades until a paste-like consistency is achieved.
  - 2.2.3. Talent transferring the minced tissue into a clean 50-milliliter tube.

## 3. Generation of a Single-Cell Suspension from Murine Tumor Tissue and Mammary Gland Tissue

- 3.1. Prepare a fresh tissue digestion cocktail immediately before tissue digestion for each sample and store it at room temperature until use [1] [2].
  - 3.1.1. WIDE: Talent preparing the tissue digestion cocktail in a container.
  - 3.1.2. TEXT ON APLAIN BACKGROUND

RPMI: 10 mL

DNase I: 400 U

Collagenase D: 25 mg

Collagenase type II: 50 mg

*Video editor: Use split screen to show both shots together*

- 3.2. Then, add 10 milliliters of the tissue digestion cocktail to each sample [1] and incubate in a shaking incubator at 37 degrees Celsius for 30 minutes at 250 rpm [2].
  - 3.2.1. Talent adding 10 milliliters of the tissue digestion cocktail to the sample.
  - 3.2.2. Talent placing the sample into the shaking incubator.
- 3.3. Add 0.5 molar EDTA acid to achieve a final concentration of 10 millimolar [1] and return the sample to the shaking incubator [2].
  - 3.3.1. Talent adding 200 microliters of 0.5 molar ethylenediaminetetraacetic acid to the sample.
  - 3.3.2. Talent placing the sample in the incubator.
- 3.4. Next, add 40 milliliters of R10 cell-washing medium to stop the reaction [1]. Filter the digested cells through a 70-micrometer filter into a clean 50-milliliter tube to remove tissue chunks [2].
  - 3.4.1. Talent adding 40 milliliters of R10 cell-washing medium to the sample.
  - 3.4.2. Talent filtering the digested cells through a 70-micrometer filter into a clean 50-milliliter tube.
- 3.5. Centrifuge the sample at 500 *g*, with acceleration 9 and deceleration 6, at 4 degrees Celsius for 10 minutes to pellet the cells [1].
  - 3.5.1. Talent placing the sample into the centrifuge.
- 3.6. Then discard the supernatant into the biological waste [1]. Disrupt the cell pellet [2], add 40 milliliters of the cell-washing medium, and centrifuge as shown earlier [3]. **NOTE:** The VO is edited for the additional shot.
  - 3.6.1. Talent discarding the supernatant into biological waste.  
**Added shot: 3.6.X Talent disrupts cell pellet**
  - 3.6.2. Talent adding 40 milliliters of the cell-washing medium to the tube.
- 3.7. Wash the cell pellet 3 times with 40 milliliters of the R10 cell-washing medium, using centrifugation to pellet the cells after each wash [1].
  - 3.7.1. Talent washing the cell pellet with 40 milliliters of R10-washing medium.
- 3.8. Then, disrupt the cell pellet [1] and resuspend the cells in 2 milliliters of PBS [2].

- 3.8.1. Talent disrupting the cell pellet by flicking the tube.
- 3.8.2. Talent adding 2 milliliters of cold PBS.
- 3.9. After diluting the 10x (*Ten-X*) RBC lysis buffer to 1x (*One-X*), add 2 volumes of the diluted buffer to the cell suspension [1]. Incubate the cell suspension at room temperature for 5 minutes while gently mixing occasionally by inverting the tube [1].
  - 3.9.1. Talent adding 2 volumes of 1 × red blood cell lysis buffer to the cell suspension.
  - 3.9.2. Talent placing the tube on the bench and gently inverting it during incubation.
- 3.10. Then, neutralize the RBC lysis buffer by adding an equal volume of R10 cell-washing medium [1].
  - 3.10.1. Talent adding an equal volume of R10 cell-washing medium to the tube.
- 3.11. Filter the cell suspension through a 30-micrometer filter into a 15-milliliter tube to remove cellular debris [1].
  - 3.11.1. Talent filtering the cell suspension through a 30-micrometer filter into a 15-milliliter tube.
- 3.12. Bring the final volume to 15 milliliters with R10 cell-washing medium [1], then centrifuge at 500 g for 10 minutes at 4 degrees Celsius [2].
  - 3.12.1. Talent adding R10 medium to adjust the volume to 15 milliliters.
  - 3.12.2. Talent placing the sample into the centrifuge.
- 3.13. After removing the supernatant, resuspend the cells in cell-washing medium to a final volume of up to 5 milliliters for counting and viability assessment [1]. Adjust the dilution as needed, then proceed with staining for flow cytometry or prepare for single-cell RNA sequencing [2].
  - 3.13.1. Talent resuspending the cells in the cell-washing medium.
  - 3.13.2. Talent adjusting the cell suspension to the desired dilution.
- 3.14. Keep the cells on ice between steps [1].
  - 3.14.1. Talent placing the cell suspension on ice between procedure steps.

#### **4. Staining the Single Cell Suspension for Flow Cytometry**

**Demonstrator:** Ryann Ray

- 4.1. Transfer the desired cells into a 5-milliliter polypropylene round-bottom tube and keep the tube on ice [1].
  - 4.1.1. Talent transfers the desired cells into a 5-milliliter polypropylene round-bottom tube and places it on ice
- 4.2. Prepare a cell staining solution by combining the desired monoclonal antibodies

conjugated to fluorescent tags with flow PBS [1-TXT].

- 4.2.1. Talent preparing the cell staining solution with the monoclonal antibodies and flow PBS. **TXT: Prepare  $(n + 1) \times 100 \mu\text{L}$  Staining Solution;  $n$  = Number of Samples**
- 4.3. Centrifuge each sample at 500 *g* for 5 minutes at 4 degrees Celsius [1]. Resuspend the cells in 100 microliters of the staining solution and the unstained controls in 1,000 microliters of flow PBS [2].
  - 4.3.1. Talent placing the samples into the centrifuge.
  - 4.3.2. Talent resuspending the cell pellet in 100 microliters of the staining solution.
- 4.4. Cover the tubes to protect them from light [1] and incubate at 4 degrees Celsius for 15 minutes [2].
  - 4.4.1. Talent covering the tubes with a light-protective cover.
  - 4.4.2. Talent placing the tubes into refrigerated incubation.
- 4.5. Next, add 1 milliliter of flow PBS to each tube containing the stained cells [1] and centrifuge at 500 *g* for 5 minutes to wash the cells [2].
  - 4.5.1. Talent adding 1 milliliter of flow phosphate-buffered saline to each stained tube.
  - 4.5.2. Talent placing the samples into the centrifuge.
- 4.6. After discarding the supernatant, disrupt the cell pellet and resuspend the cells in 1 milliliter of flow PBS [1]. Then repeat the centrifugation and wash procedure 3 times [2].
  - 4.6.1. Talent resuspending the cells in 1 milliliter of flow PBS.
  - 4.6.2. Talent placing the samples into the centrifuge.
- 4.7. For fixing the cells or performing an intracellular stain, add 200 microliters of the permeabilization and fixation solution to each sample to allow for intranuclear staining [1].
  - 4.7.1. Talent adding 200 microliters of the permeabilization and fixation solution to each sample.
- 4.8. Cover the tubes with aluminum foil to protect them from light [1] and incubate at 4 degrees Celsius for at least 20 minutes or overnight if the samples will be analyzed the following day [2].
  - 4.8.1. Talent covering the tubes with aluminum foil.
  - 4.8.2. Talent placing the covered tubes into refrigerated incubation.
- 4.9. Centrifuge the cells at 500 *g* for 5 minutes after fixation [1] and resuspend them in permeabilization buffer diluted in water [2].

- 4.9.1. Talent placing the samples into the centrifuge.
- 4.9.2. Talent resuspending the cells in 1 × permeabilization buffer.
- 4.10. Next, prepare the secondary or intracellular stain at the desired dilution in permeabilization buffer [1]. Stain the samples with the prepared solution [2] and place the covered tubes at 4 degrees Celsius for 15 minutes [3].
  - 4.10.1. Talent preparing the secondary or intracellular staining solution.
  - 4.10.2. Talent adding the staining solution to the samples.
  - 4.10.3. Talent placing the covered tubes into a covered ice bucket.
- 4.11. Add 1 milliliter of permeabilization buffer to each stained tube, then centrifuge as shown earlier [1].
  - 4.11.1. Talent adding 1 milliliter of 1 × permeabilization buffer to each stained tube.
- 4.12. Disrupt the cell pellet, resuspend in 1 milliliter of permeabilization buffer [1], and centrifuge as demonstrated earlier [2].
  - 4.12.1. Talent resuspending the cells in 1 milliliter of 1 × permeabilization buffer.
  - 4.12.2. Talent placing the samples into the centrifuge.
- 4.13. After washing the cells three times, resuspend the cells in 200 microliters of permeabilization buffer [1] and keep the samples on ice until they are ready to be analyzed on a flow cytometer [2].
  - 4.13.1. Talent resuspending the cells in 200 microliters of 1 × permeabilization buffer.
  - 4.13.2. Talent placing the samples on ice for storage before flow cytometry.
- 4.14. If the cells are not fixed, resuspend them in 200 microliters of flow PBS [1] and keep the samples on ice until they are ready for flow cytometry analysis [2].
  - 4.14.1. Talent resuspending the cells in 200 microliters of flow phosphate-buffered saline.
  - 4.14.2. Talent placing the samples on ice before flow cytometry.
- 4.15. Remove debris and dead cells for single-cell RNA analysis, then assess RNA quality and generate sequencing libraries [1].
  - 4.15.1. Talent assessing ribonucleic acid quality on the computer on nanometer display

# Results

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## 5. Results

5.1. For direct comparison, dissociation was carried out concurrently using a commercial kit [1]. The dissociation protocol recovered more viable cells [2] than the commercial kit did, both before and after dead-cell removal [3]. The fixation-first protocol yielded  $1.5 \times 10^7$  cells per milliliter without viability measurement [4], whereas the dissociation protocol yielded  $8.6 \times 10^6$  cells per milliliter from tissue with 94% starting viability [5].

5.1.1. LAB MEDIA: Figure 2

5.1.2. LAB MEDIA: Figure 2A,B. *Video editor: Highlight the image labeled "This Dissociation".*

5.1.3. LAB MEDIA: Figure 2C,D. *Video editor: Highlight the image labeled "This Dissociation"*

5.1.4. LAB MEDIA: Figure 2E,F. *Video editor: Highlight the image labelled "Chop Fix"*

5.1.5. LAB MEDIA: Figure 2E,F. *Video editor: Highlight the image labelled "This protocol"*

5.2. Flow cytometry analysis identified epithelial cells [1], fibroblasts [2], lymphocytes [3], regulatory T cells [4], macrophages [5], dendritic cells [6], and neutrophils, demonstrating preservation of diverse cell populations after dissociation [7].

5.2.1. LAB MEDIA: Figure 3A.

5.2.2. LAB MEDIA: Figure 3B.

5.2.3. LAB MEDIA: Figure 3C.

5.2.4. LAB MEDIA: Figure 3F.

5.2.5. LAB MEDIA: Figure 3G.

5.2.6. LAB MEDIA: Figure 3H.

5.2.7. LAB MEDIA: Figure 3I.

5.3. Live-cell dissociation produced high-quality single-cell RNA sequencing libraries with clear barcode knee bends [1], distinct cell clusters, and higher library quality [2] than the commercial fixation-first dissociation protocol for mammary gland and tumor-containing samples [3].

5.3.1. LAB MEDIA: Figure 4A and C. *Video editor: Highlight the top panel*

- 5.3.2. LAB MEDIA: Figure 4A and C. *Video editor: Highlight the lower panel.*
- 5.3.3. LAB MEDIA: Figure 4B and D
- 5.4. Integrated single-cell RNA sequencing identified comparable immune and stromal populations between samples [1] and an additional tumor-cell population in BALB (*Balb*) NeuT (*Neu-T*) tissue [2].
  - 5.4.1. LAB MEDIA: Figure 4E.
  - 5.4.2. LAB MEDIA: Figure 4F. *Video editor: Highlight the black cluster labeled "Tumor cells".*
  - 5.4.3. LAB MEDIA: Figure 4F. *Video editor: Highlight the clusters labeled "Macrophages", "T cells", and "Areg Epi".*
- 5.5. Neither the BALB nor the BALB NeuT libraries contained significant numbers of neutrophils [1]. However, NeuT mice in a BALB/PWK (*Balb-P-W-K*) background harbored a cell cluster expressing neutrophil markers [2].
  - 5.5.1. LAB MEDIA: Figure 5A and B
  - 5.5.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the row labeled "PMN"*

1. Mammary  
Pronunciation link: <https://www.merriam-webster.com/dictionary/mammary>  
IPA: /'mæməri/  
Phonetic Spelling: MAM·uh·ree
2. Necrotic  
Pronunciation link: <https://www.merriam-webster.com/dictionary/necrotic>  
IPA: /nə'krɑ:tɪk/  
Phonetic Spelling: nuh·KROT·ik
3. Conical  
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>  
IPA: /'kɑ:nɪkəl/  
Phonetic Spelling: KON·ih·kuhl
4. Petri  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>  
IPA: /'pi:tri/  
Phonetic Spelling: PEE·tree
5. Murine  
Pronunciation link: <https://www.merriam-webster.com/dictionary/murine>  
IPA: /'mjʊrən/  
Phonetic Spelling: MYUR·ine
6. DNase  
Pronunciation link: No confirmed link found  
IPA: /'diː,ɛn,eɪs/  
Phonetic Spelling: DEE·EN·ays
7. Collagenase  
Pronunciation link: <https://www.merriam-webster.com/medical/collagenase>  
IPA: /kə'lædʒəˌneɪs/  
Phonetic Spelling: kuh·LAJ·uh·nays
8. Molar  
Pronunciation link: <https://www.merriam-webster.com/dictionary/molar>  
IPA: /'moʊlər/  
Phonetic Spelling: MOH·ler
9. Ethylenediaminetetraacetic  
Pronunciation link: No confirmed link found  
IPA: /,ɛθəliːn,dɑɪəˌmiːn,tetɹə'si:tɪk/  
Phonetic Spelling: eth·uh·leen·dye·uh·meen·tet·ruh·uh·SEE·tik
10. Centrifuge  
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>  
IPA: /'sentrəˌfjuːdʒ/  
Phonetic Spelling: SEN·truh·fyooj
11. Supernatant  
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>  
IPA: /,suːpərˈneɪtənt/  
Phonetic Spelling: soo·per·NAY·tuhnt

12. Resuspend  
Pronunciation link: No confirmed link found  
IPA: /ˌriːsəˈspend/  
Phonetic Spelling: ree·suh·SPEND
13. Viability  
Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>  
IPA: /ˌvaɪəˈbɪləti/  
Phonetic Spelling: vy·uh·BIL·uh·tee
14. Cytometry  
Pronunciation link: <https://www.merriam-webster.com/medical/cytometry>  
IPA: /sarˈtɑːmətri/  
Phonetic Spelling: sy·TOM·uh·tree
15. Polypropylene  
Pronunciation link: <https://www.merriam-webster.com/dictionary/polypropylene>  
IPA: /ˌpɑːliˈproʊpəˌliːn/  
Phonetic Spelling: pol·ee·PROH·puh·leen
16. Monoclonal  
Pronunciation link: <https://www.merriam-webster.com/dictionary/monoclonal>  
IPA: /ˌmɑːnəˈkloʊnəl/  
Phonetic Spelling: mon·uh·KLOH·nuhl
17. Antibodies  
Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>  
IPA: /ˈæntɪˌbɑːdiz/  
Phonetic Spelling: AN·tee·bod·eez
18. Conjugated  
Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugate>  
IPA: /ˈkɑːndʒəˌgeɪtɪd/  
Phonetic Spelling: KON·juh·gay·tid
19. Fluorescent  
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>  
IPA: /flʊˈresənt/  
Phonetic Spelling: floor·ES·uhnt
20. Permeabilization  
Pronunciation link: No confirmed link found  
IPA: /ˌpɜːrmɪəbələˈzeɪʃən/  
Phonetic Spelling: per·mee·uh·buh·luh·ZAY·shuhn
21. Intranuclear  
Pronunciation link: No confirmed link found  
IPA: /ˌɪntrəˈnuːkliər/  
Phonetic Spelling: in·truh·NOO·klee·er
22. Intracellular  
Pronunciation link: <https://www.merriam-webster.com/dictionary/intracellular>  
IPA: /ˌɪntrəˈseljələr/  
Phonetic Spelling: in·truh·SEL·yuh·ler

23. Cytometer  
 Pronunciation link: No confirmed link found  
 IPA: /saɪˈtəːmɪtər/  
 Phonetic Spelling: sy·TOM·ih·ter
24. Ribonucleic  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ribonucleic>  
 IPA: /ˌraɪboʊnuːˈkliːɪk/  
 Phonetic Spelling: rye·boh·noo·KLEE·ik
25. Dissociation  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociation>  
 IPA: /dɪˌsoʊsiˈeɪʃən/  
 Phonetic Spelling: dih·soh·see·AY·shuhn
26. Epithelial  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/epithelial>  
 IPA: /ˌɛpəˈθiːliəl/  
 Phonetic Spelling: ep·uh·THEE·lee·uhl
27. Fibroblasts  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fibroblast>  
 IPA: /ˈfaɪbrəˌblæsts/  
 Phonetic Spelling: FY·bruh·blasts
28. Lymphocytes  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/lymphocyte>  
 IPA: /ˈlɪmfəˌsaɪts/  
 Phonetic Spelling: LIM·fuh·sytes
29. Macrophages  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/macrophage>  
 IPA: /ˈmækrəˌfeɪdʒɪz/  
 Phonetic Spelling: MAK·ruh·fay·jiz
30. Dendritic  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dendritic>  
 IPA: /denˈdɹɪtɪk/  
 Phonetic Spelling: den·DRIT·ik
31. Neutrophils  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/neutrophil>  
 IPA: /ˈnuːtrəˌfɪlz/  
 Phonetic Spelling: NOO·truh·filz
32. Stromal  
 Pronunciation link: <https://www.merriam-webster.com/medical/stromal>  
 IPA: /ˈstroʊməl/  
 Phonetic Spelling: STROH·muhl
33. Sequencing  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sequence>  
 IPA: /ˈsiːkwənsɪŋ/  
 Phonetic Spelling: SEE·kwuhn·sing

34. Barcode

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

IPA: /'bɑːr,kəʊd/

Phonetic Spelling: BAR·kohd

35. BALB

Pronunciation link: No confirmed link found

IPA: /bælb/

Phonetic Spelling: BALB

36. NeuT

Pronunciation link: No confirmed link found

IPA: /nu:'ti:/

Phonetic Spelling: noo·TEE

37. Neutrophil

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

IPA: /'nu:trəˌfɪl/

Phonetic Spelling: NOO·truh·fil