

**Submission ID #: 71138**

**Scriptwriter Name: Sri Lakshmi Priya**

**Project Page Link: <https://review.jove.com/account/file-uploader?src=21697138>**

**Title: Clinically Relevant Transplantable Tumor Models for Studying Metastatic Ovarian Cancer and Therapeutic Response in Mouse**

**Authors and Affiliations:**

Megha J Pandya<sup>1\*</sup>, Fatimah Alfaran<sup>1\*</sup>, Ayokunnumi Ogunsanya<sup>1\*</sup>, Swethakumar Basavarajaiah<sup>1</sup>, Achuth Padmanabhan<sup>1,2</sup>

<sup>1</sup>Department of Biological Sciences, University of Maryland, Baltimore County

<sup>2</sup>University of Maryland Greenebaum Comprehensive Cancer Center

\* These authors contributed equally

**Corresponding Authors:**

Achuth Padmanabhan      achuth1@umbc.edu

**Email Addresses for All Authors:**

|                           |                   |
|---------------------------|-------------------|
| Megha J Pandya            | meghajp1@umbc.edu |
| Fatimah Alfaran           | tp47410@umbc.edu  |
| Ayokunnumi Ogunsanya      | aogunsa2@umbc.edu |
| Swethakumar Basavarajaiah | swethab1@umbc.edu |
| Achuth Padmanabhan        | achuth1@umbc.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? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

**Enter Yes or No.**

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

**Enter make and model of microscope.**

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

**Scope shot: 2.8.1**

*Videographer: Please film the above-mentioned scope shot using a scope kit.*

**NOTE: Please check the videographer file for the scope shot**

**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? **Yes**

**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: 39

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. **Achuth Padmanabhan:** Our research focuses on identifying the molecular factors that drive ovarian cancer progression and therapeutic resistance.
  - 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. **Fatimah Alfaran:** These models can be used to study how various factors impact ovarian cancer progression and test new therapeutic strategies.
  - 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?~~

- 1.3. **Fatimah Alfaran:** This step-by-step protocol enables researchers to develop in vivo models to investigate ovarian cancer metastasis and therapeutic response.
  - 1.3.1. 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.~~

- 1.4. **Megha Pandya:** The greatest challenge in this protocol is inoculating cells into the ovarian bursa with proper technique to avoid leakage.
  - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.5. **Ayokunnumi Ogunsanya:** Following this procedure, researchers can perform in vivo imaging approaches for quantitative, longitudinal assessment of tumor burden.
  - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer's Notes: Did each interview question at least twice. Most of the slates should be accurate. A couple times talent said they were doing 1 step but did 2 or 3 in the same shot. Had an issue with my still camera, so for one of the speakers in the interview I had to take the still with my Blackmagic camera. Those photos are in .dng format. If you need me to convert them to jpeg or PNG, let me know.

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

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Maryland

# Protocol

---

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

## **2. Preparation of Ovarian Cancer Cell Suspension**

**Demonstrator:** Ayokunnumi Ogunsanya

- 2.1. To begin, obtain ovarian cancer cell culture. **[1]** Once the cells reach 80 to 85% confluency, aspirate the medium from the tissue culture plate. **[2]**
  - 2.1.1. WIDE: Talent sitting with tissue culture plate on the workbench.  
***Videographer:** Please obtain CU shot as well for this shot.*
  - 2.1.2. Talent aspirating the media from the tissue culture plate.
- 2.2. Wash the adherent cells twice with sterile 1X PBS without calcium and magnesium. **[1]** Aspirate the PBS from the tissue culture plate. **[2]**
  - 2.2.1. Talent adding PBS to the tissue culture plate.
  - 2.2.2. Talent aspirating the PBS from the tissue culture plate.
- 2.3. Add 0.05% Trypsin-EDTA to the tissue culture plate **[1]**.
  - 2.3.1. Talent adding Trypsin-EDTA to the tissue culture plate.
- 2.4. Transfer the plate with cells to the 37 degrees Celsius water-jacketed carbon dioxide incubator used for tissue culture and incubate for 1 to 5 minutes **[1]**.
  - 2.4.1. Talent placing the tissue culture plate into a 37 degrees Celsius water-jacketed carbon dioxide incubator and closing the door.
- 2.5. Once the cells are detached, add FBS-containing media to the tissue culture plate at a ratio of 6 milliliters of media for every 1 milliliter of trypsin used **[1]**. Gently pipette up and down to dislodge the cells from the tissue culture plate and dissociate them into a single-cell suspension **[2]**.
  - 2.5.1. Talent adding FBS-containing media to the tissue culture plate.
  - 2.5.2. Talent gently pipetting the cell suspension up and down.
- 2.6. Collect the cells in a conical tube **[1]** and centrifuge at 400 g for 5 minutes at room temperature **[2]**.
  - 2.6.1. Talent transferring the cell suspension into a conical tube.
  - 2.6.2. Talent placing the conical tube into the centrifuge.
- 2.7. After removing the trypsin-containing supernatant, resuspend the cell pellet in fresh FBS-containing cell culture media **[1]**.

- 2.7.1. Talent resuspending the cell pellet in fresh FBS-containing cell culture media.
- 2.8. Using a hemocytometer, count the trypan blue-stained cells [1].
  - 2.8.1. SCOPE: Shot of trypan blue-stained cells in the hemocytometer. **NOTE: Please check the videographer file.**
- 2.9. Reconstitute the cells in a defined volume of sterile 1X PBS at pH 7.4 to achieve the desired cell concentration [1].
  - 2.9.1. Talent adding PBS to the cell pellet.
- 2.10. Keep the cell suspension on ice until it is ready for injection into mice. [1]
  - 2.10.1. Talent placing the tube containing the cell suspension on ice.

### **3. Intraperitoneal Injection of Ovarian Cancer Cells into Mice**

**Demonstrator:** Fatimah Alfaran

- 3.1. Using a 1000 microliter micropipette, gently resuspend the cells and ensure that the cell suspension remains dissociated [1].
  - 3.1.1. Talent gently resuspending the cells using a 1000 microliter micropipette.
- 3.2. Draw 500 microliters of the cell suspension into a 1 milliliter syringe [1]. Attach a 21-gauge by 1-inch needle to the syringe and ensure there are no air bubbles [2].
  - 3.2.1. Talent drawing the cell suspension into a 1 milliliter syringe.
  - 3.2.2. Talent attaching a 21-gauge by 1-inch needle to the syringe.
- 3.3. After removing the mouse from the cage, place it on a rough or wired surface for restraint [1].
  - 3.3.1. Talent placing the mouse on a rough or wired surface.
- 3.4. Using the non-dominant hand, restrain the mouse by gently scruffing the loose skin at the base of the mouse's neck [1].
  - 3.4.1. Talent restraining the mouse by scruffing the loose skin at the base of the neck.
- 3.5. After gently lifting the mouse, position it with the ventral side facing upward. [1] Secure the tail with fingers [2].
  - 3.5.1. Talent lifting the mouse and positioning it with the ventral side facing upward.
  - 3.5.2. Close-up of the tail being secured with the fingers.
- 3.6. Draw an imaginary line across the middle of the abdomen [1]. Define an imaginary triangular region between the midline and the two nipples in the lower right quadrant [2]. Locate the injection site in the middle of this imaginary triangle [3].

- 3.6.1. Close-up of the restrained mouse with the talent using a finger to trace across the midpoint of the abdomen from left to right.
- 3.6.2. Close-up of the talent sequentially pointing to the abdominal midline and the two nipples in the lower right quadrant to establish the boundaries of the injection triangle.
- 3.6.3. Close-up of the talent placing a fingertip over the center of the identified triangular region to mark the injection site.
- 3.7. Tilt the mouse with its head slightly toward the ground and lower than its hind end [1].
  - 3.7.1. Talent tilting the mouse head, positioned lower than the hind end.
- 3.8. After cleaning the injection area with a sterile alcohol swab, using the dominant hand, insert the needle into the injection site at an angle of 45 degrees or lower [1]. Advance the needle 0.5 inch and no more than half of its length [2].
  - 3.8.1. Talent inserting the needle into the injection site at an angle of 45 degrees or lower.
  - 3.8.2. Talent advancing the needle to a depth of 0.5 inch.
- 3.9. Dispense the cells slowly and steadily into the abdominal cavity of the mouse [1].
  - 3.9.1. Talent slowly dispensing the cell suspension into the abdominal cavity of the mouse.
- 3.10. After withdrawing the needle at the same angle used for insertion, immediately place a sterilized gauze over the injection site [1].
  - 3.10.1. Talent placing a sterilized gauze over the injection site.
- 3.11. Ear tag the mouse before returning it to the cage [1].
  - 3.11.1. Talent ear tagging the mouse.

#### **4. Intrabursal Transplantation of Ovarian Cancer Cells and Postoperative Monitoring**

**Demonstrator:** Megha Pandya and Swethakumar Basavarajaiah

- 4.1. After anesthetizing the mouse, confirm anesthesia by checking for the absence of the toe pinch reflex [1-TXT].
  - 4.1.1. Talent gently performing a toe pinch test on the mouse with no withdrawal response observed. **TXT: Anesthesia: 4% isoflurane, 3.5 L/min O<sub>2</sub>**
- 4.2. Transfer the mouse onto a sterile surgical drape placed on top of a rodent heating pad maintained at 37 degrees Celsius [1]. Quickly place a nose cone and maintain anesthesia throughout the procedure, confirming anesthetic depth by toe pinch every 15 minutes [2-TXT].

- 4.2.1. Talent transferring the anesthetized mouse onto the sterile surgical drape on the rodent heating pad.
- 4.2.2. Talent placing the nose cone on the mouse. **TXT: Anesthesia: 1.5% isoflurane, 98.5% O<sub>2</sub>**
- 4.3. Ensure the breathing rate remains slow and smooth during anesthesia by adjusting the isoflurane dosage as needed [1].
  - 4.3.1. Close-up of the mouse's thorax showing slow, regular breathing movements.
- 4.4. Position the mouse with the side in which the ovarian cancer cells are to be transplanted intrabursally facing up [1].
  - 4.4.1. Talent positioning the mouse with the transplantation side facing up.
- 4.5. After applying eye ointment to both eyes of the mouse, using a sterile surgical drape, cover the mouse so that only the lower half of the body is revealed [1].
  - 4.5.1. Talent covering the mouse with a sterile surgical drape, leaving only the lower half of the body exposed.
- 4.6. After washing the hands with soap and putting on a fresh pair of sterile surgical gloves, use a sterile cotton tip applicator to apply betadine liquid at the surgical site in a clockwise circular motion from the inside out [1]. Next, use a sterile alcohol swab to clean the same area in a similar fashion and repeat this procedure 2 more times [2].
  - 4.6.1. Talent applying betadine liquid at the surgical site with a sterile cotton tip applicator in a clockwise circular motion from the inside out.
  - 4.6.2. Talent cleaning the same area with a sterile alcohol swab.
- 4.7. Once the alcohol is dried, locate the site approximately 1.5 centimeters above the hind leg and 5 millimeters from the rib cage of the mouse [1]. Using curved forceps, lift the skin at this site [2], and, with fine-tipped scissors, make a 5-millimeter vertical incision [3].
  - 4.7.1. Talent indicating the incision site above the hind leg and near the rib cage.
  - 4.7.2. Talent lifting the skin with curved forceps.
  - 4.7.3. Talent making a vertical incision with fine-tipped scissors.
- 4.8. After holding the skin on one side using fine-tip microforceps, make an incision in the subcutaneous tissue at the same site [1].
  - 4.8.1. Talent holding the skin on one side with fine-tip microforceps while making an incision in the subcutaneous tissue.
- 4.9. Once the visible peritoneal layer is identified under the subcutaneous tissue, using fine-tipped, curved, serrated forceps, hold the peritoneal layer [1]. Then, with fine-tipped scissors, make an incision approximately 2.5 millimeters long [2].
  - 4.9.1. Talent holding the peritoneal layer with fine-tipped, curved, serrated forceps.

- 4.9.2. Talent making a 2.5-millimeter incision with fine-tipped scissors.
- 4.10. Using the serrated forceps, hold the side of the peritoneal incision flap [1]. After locating the ovarian fat pad with fine forceps, gently pull out a small portion of it along with the ovary through the incision [2].
  - 4.10.1. Talent holding the side of the peritoneal incision flap with serrated forceps.
  - 4.10.2. Talent using fine forceps to gently pull a small portion of the ovarian fat pad and ovary through the incision.
- 4.11. Use dissecting forceps to hold the fat pad so that the ovary faces up and remains immobile [1].
  - 4.11.1. Talent holding the fat pad with dissecting forceps while the ovary faces up.
- 4.12. Insert the needle with the bevel facing up into the ovarian bursa from the junction of the fallopian tube and ovary, ensuring that the entire bevel enters the bursa before pressing the plunger [1].
  - 4.12.1. Talent inserting the needle into the ovarian bursa with the bevel facing up.
- 4.13. Gently push the plunger and transfer the contents of the syringe into the ovarian bursa [1]. After quickly removing the needle, wait for 5 seconds, then gently return the ovary to the peritoneal cavity [2].
  - 4.13.1. Talent pushing the plunger to transfer the cell suspension into the ovarian bursa.
  - 4.13.2. Talent gently returning the ovary into the peritoneal cavity.
- 4.14. Close the peritoneum using a 6-0 (six-oh) absorbable monofilament suture with 1 or 2 simple interrupted surgical stitches [1]. Then, close the skin incision with a simple running stitch, ensuring there is no bleeding from the incision site [2].
  - 4.14.1. Talent closing the peritoneum with a 6-0 absorbable monofilament suture.
  - 4.14.2. Talent closing the skin incision with the 6-0 absorbable monofilament suture.
- 4.15. Apply a triple-antibiotic ointment containing Bacitracin, Neomycin, and Polymyxin B at the incision site after closure [1].
  - 4.15.1. Talent applying triple antibiotic ointment at the closed incision site.
- 4.16. Administer Carprofen at 15 milligrams per kilogram subcutaneously to the mouse for pain relief [1].
  - 4.16.1. Talent administering Carprofen subcutaneously to the mouse.
- 4.17. After ear tagging, transfer the mouse to a fresh cage [1]. Place one-half of the cage on a heat pad [2].
  - 4.17.1. Talent transferring the ear-tagged mouse to a fresh cage.
  - 4.17.2. Talent placing one-half of the cage on a heat pad.

4.18. At the desired experimental time points following D-luciferin administration, acquire dorsal and ventral bioluminescence images of the mouse using the IVIS (*eye-vis*) imaging system [1]. Analyze the acquired images to quantify tumor burden over time [2].

4.18.1. Talent acquiring a bioluminescence image of the mouse using the IVIS imaging system.

4.18.2. Talent analyzing the images on a computer.

# Results

---

## 5. Results

5.1. Intraperitoneal injection of 5 million OVCAR8-luciferase (*Ovcar-eight-luciferase*) cells into Foxn1 (*Fox-N-one*) nude mice established metastatic tumors in the peritoneal cavity with a 100 percent take rate [1].

5.1.1. LAB MEDIA: Figure 4C.

5.2. The tumors progressed rapidly, resulting in a large tumor burden [1] and ascites [2].

5.2.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the enlarged belly outlined with the dashed circle*

5.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the area pointed to by the arrow labeled "Omental tumor"*

5.3. *In vivo* imaging revealed high tumor burden within 29 days after intraperitoneal inoculation of 5 million OVCAR8-luciferase cells in Foxn1 nude mice [1].

5.3.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the row labeled "Day 29"*

5.4. Tumor burden was significantly lower in mice injected with 1 million OVCAR8-luciferase cells [1] than in mice injected with 5 million cells [2].

5.4.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the panel labeled "1 x 10<sup>6</sup> OVCAR8-Luciferase cells"*

5.4.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the panel labeled "5 x 10<sup>6</sup> OVCAR8-Luciferase cells"*

5.5. The tumor burden in mice injected with 1 million cells at 49 days post-injection [1] was lower than that observed at 29 days post-injection in mice injected with 5 million cells [2].

5.5.1. LAB MEDIA: Figure 4D. *Video editor: Highlight the row labeled "Day 49"*

5.5.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the row labeled "Day 29"*

5.6. Mice injected with 5 million cells survived for fewer days [1] than mice injected with 1 million cells [2].

5.6.1. LAB MEDIA: Figure 4E. *Video editor: Highlight the black survival line*

5.6.2. LAB MEDIA: Figure 4E. *Video editor: Highlight the red survival line*

5.7. IVIS imaging on day 1 post-injection confirmed successful intrabursal transplantation of OVCA420 (*Ovca-four-twenty*) cells [1].

5.7.1. LAB MEDIA: *Figure 5A. Video editor: Highlight the light-emitting signal visible in both mice on Day 1*

- 5.8. In vivo imaging at day 28 post intrabursal injection demonstrated a higher tumor burden in mice inoculated with ZNF217 (*Z-N-F-two-one-seven*) overexpressing OVCA420 cells [1] compared to control OVCA420 cells [2].
- 5.8.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the mouse labeled “OVCA420-ZNF217” in the Day 28 row*
  - 5.8.2. LAB MEDIA: Figure 5B. *Video editor: Highlight the mouse labeled “OVCA420” in the Day 28 row*
- 5.9. Tumor burden increased over time in mice inoculated with ZNF217-overexpressing OVCA420 cells [1] and remained higher than in control OVCA420 mice [2].
- 5.9.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the red line labeled “OVCA420-ZNF217”*
  - 5.9.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the blue line labeled “OVCA420”*

**Pronunciation guides:**

1. Confluency  
Pronunciation link: <https://www.merriam-webster.com/dictionary/confluency>  
IPA: /kən'flu:ənsi/  
Phonetic Spelling: kuhn·FLOO·uhn·see
2. Adherent  
Pronunciation link: <https://www.merriam-webster.com/dictionary/adherent>  
IPA: /əd'hɪrənt/  
Phonetic Spelling: uh·DHEER·uhnt
3. Trypsin-EDTA  
Pronunciation link: <https://www.howtopronounce.com/trypsin>  
IPA: /'trɪpsɪn/  
Phonetic Spelling: TRIP·sin E-D-T-A
4. Dissociate  
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissociate>  
IPA: /dɪ'soʊʃi,et/, /dɪ'soʊsi,et/  
Phonetic Spelling: dih·SOH·shee·ayt; dih·SOH·see·ayt
5. Centrifuge  
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>  
IPA: /'sentrə,fju:dʒ/  
Phonetic Spelling: SEN·truh·fyooj
6. Supernatant  
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>  
IPA: /,su:pər'nextənt/  
Phonetic Spelling: SOO·per·NAY·tuht

7. Resuspend  
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>  
IPA: /ˌriːsəˈspend/  
Phonetic Spelling: ree-suh-SPEND
8. Hemocytometer  
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemocytometer>  
IPA: /ˌhiːmoʊˌsaɪˈtəːmətər/  
Phonetic Spelling: HEE-moh-sy-TOM-uh-ter
9. Trypan  
Pronunciation link: <https://www.howtopronounce.com/trypan>  
IPA: /ˈtraɪpæn/  
Phonetic Spelling: TRY-pan
10. Intraperitoneal  
Pronunciation link: <https://www.merriam-webster.com/dictionary/intraperitoneal>  
IPA: /ˌɪntrəˌperɪtəˈniːəl/  
Phonetic Spelling: IN-truh-per-ih-tuh-NEE-uhl
11. Micropipette  
Pronunciation link: <https://www.merriam-webster.com/dictionary/micropipette>  
IPA: /ˌmaɪkroʊpaɪˈpɛt/  
Phonetic Spelling: MY-kroh-py-PET
12. Ventral  
Pronunciation link: <https://www.merriam-webster.com/dictionary/ventral>  
IPA: /ˈvɛntrəl/  
Phonetic Spelling: VEN-truhl
13. Abdomen  
Pronunciation link: <https://www.merriam-webster.com/dictionary/abdomen>  
IPA: /ˈæbdəmən/  
Phonetic Spelling: AB-duh-muhn
14. Anesthetizing  
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>  
IPA: /əˈnɛsθəˌtaɪz/  
Phonetic Spelling: uh-NES-thuh-tyze
15. Isoflurane  
Pronunciation link: <https://www.howtopronounce.com/isoflurane>  
IPA: /ˌaɪsoʊˈflʊreɪn/  
Phonetic Spelling: EYE-soh-FLOOR-ayn
16. Intrabursal  
Pronunciation link: <https://www.howtopronounce.com/intrabursal>  
IPA: /ˌɪntrəˈbɜːsəl/  
Phonetic Spelling: IN-truh-BUR-suhl
17. Betadine  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Betadine>

IPA: /'beɪtəˌdaɪn/

Phonetic Spelling: BAY·tuh·dyne

18. Subcutaneous

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

IPA: /ˌsʌbkjuːˈteɪniəs/

Phonetic Spelling: sub·kyoo·TAY·nee·uhs

19. Peritoneal

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

IPA: /ˌperɪtəˈniːəl/

Phonetic Spelling: per·ih·tuh·NEE·uhl

20. Microforceps

Pronunciation link: <https://www.howtopronounce.com/forceps>

IPA: /ˈfɔːrseps/

Phonetic Spelling: MY·kroh·FOR·seps

21. Ovary

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

IPA: /ˈoʊvəri/

Phonetic Spelling: OH·vuh·ree

22. Bursa

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

IPA: /ˈbɜːsə/

Phonetic Spelling: BUR·suh

23. Fallopian

Pronunciation link: <https://www.merriam-webster.com/dictionary/Fallopian%20tube>

IPA: /fəˈlɒpiən/

Phonetic Spelling: fuh·LOH·pee·uhn

24. Monofilament

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

IPA: /ˌmɒːnoʊˈfɪləmənt/

Phonetic Spelling: MON·oh·FIL·uh·muhnt

25. Bacitracin

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

IPA: /ˌbæsəˈtreɪsɪn/

Phonetic Spelling: BASS·uh·TRAY·sin

26. Neomycin

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

IPA: /ˌniːoʊˈmaɪsɪn/

Phonetic Spelling: NEE·oh·MY·sin

27. Polymyxin

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

IPA: /ˌpɑːliˈmɪksɪn/

Phonetic Spelling: POL·ee·MIK·sin

28. Carprofen

Pronunciation link: <https://www.howtopronounce.com/carprofen>

IPA: /'kɑ:rproufən/

Phonetic Spelling: KAR·proh·fen

29. Bioluminescence

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

IPA: /ˌbaɪoʊˌluːməˈnesəns/

Phonetic Spelling: BY·oh·loo·muh·NESS·uhns

30. Luciferase

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

IPA: /ˌluːsɪfəˈreɪs/

Phonetic Spelling: LOO·sih·fuh·rays

31. Ascites

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

IPA: /əˈsaɪtiːz/

Phonetic Spelling: uh·SY·teez

32. Inoculation

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

IPA: /ɪˌnoʊˌkjəˈleɪʃən/

Phonetic Spelling: ih·NOK·yuh·LAY·shuhn