

Submission ID #: 71559

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21825543>

Title: Quantifying Embryonic Lethality Rate in *Caenorhabditis elegans*

Authors and Affiliations:

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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?

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.**

N/A

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 shots: 4.3.2, 5.1.2, 5.2.2, 5.4.1, 5.6.2, 5.7.2, 5.9.2

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

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**, all done

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. **Yes**

Current Protocol Length

Number of Steps: 29

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. **Andreana Nourie:** Our research focuses on measuring embryonic lethality in *Caenorhabditis elegans* and determining how strongly a perturbation affects development.
 - 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. **Andreana Nourie:** This protocol can be applied to assess embryonic lethality caused by genetic mutations, RNAi knockdown, drug treatment, nutritional supplementations, or environmental perturbations.
 - 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. **Garrick Tam:** The most important considerations when performing this protocol are transferring sufficient embryos and accurately distinguishing eggs from debris.
 - 1.3.1. 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?~~

- 1.4. **Andreana Nourie:** Researchers can also note the developmental stage at which embryos arrest while performing this procedure.
 - 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. **Garrick Tam:** Incorporating time-restricted egg laying and time-course hatching analyses could improve resolution and distinguish embryonic lethality from developmental delay.
 - 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.

Testimonial Questions (OPTIONAL):

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

~~What value do you expect this video article to have for other researchers working in your field?~~

- 1.6. **Andreana Nourie, Graduate Student at the University of New Mexico**: (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.

~~How does the video format of your article help you to share your work with the broader scientific community?~~

- 1.7. **Andreana Nourie, Graduate Student at the University of New Mexico**: (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.

Protocol

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

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

2. *C. elegans* Maintenance and Preparation of Maintenance Plates

Demonstrator: Andreana Nourie

2.1. To begin, gather the required reagents [1]. Prepare 1 liter of Nematode Growth Medium or NGM (*N-G-M*) in a 2-liter bottle [2]. Allow the medium to cool to 55 degrees Celsius in a water bath [3]. After placing the bottle on a stir plate, aseptically, add the required reagents [4]. Pour approximately 25 milliliters of medium into each 100-millimeter plate [5].

2.1.1. WIDE: Talent arranging the reagents on the workbench. *Videographer: Please obtain CU shot as well for this shot.*

2.1.2. TEXT ON A PLAIN BACKGROUND:

Preparation of NGM:

NaCl - 3 g

Bacteriological peptone - 2.5 g

Agar - 17 g

Distilled water - 975 mL

Add stir bar and autoclave for 20 min at 121 °C

2.1.3. Talent placing the bottle containing the medium into a water bath.

2.1.4. TEXT ON A PLAIN BACKGROUND:

Potassium phosphate buffer - 25 mL

1 M MgSO₄ – 1 mL

1 M CaCl₂ – 1 mL

5 mg/mL cholesterol – 1 mL

2.1.5. Talent pouring the medium into 100-millimeter plates.

2.2. Once the 1-liter Erlenmeyer flask is aseptically filled with 200 milliliters of LB (*L-B*) medium, using a sterile inoculating loop, inoculate the medium with a single colony of *E. coli* OP50 (*O-P-fifty*) [1]. Place the flask on an orbital shaker at 200 rpm and incubate overnight at 37 degrees Celsius [2].

2.2.1. Talent inoculating the LB medium in a 1-liter Erlenmeyer flask with a single *E. coli* OP50 colony using a sterile inoculating loop.

2.2.2. Talent placing the flask onto an orbital shaker.

- 2.3. Transfer the *E. coli* OP50 culture into autoclaved centrifuge bottles [1]. After centrifuging at room temperature for 30 minutes at 3500 g, resuspend the bacterial pellet in one-twentieth of the original volume [2]. Seed each 100-millimeter NGM plate with 1 to 1.5 milliliters of the concentrated culture and incubate overnight at room temperature to allow the bacteria to grow [3].
 - 2.3.1. Talent transferring the *E. coli* OP50 culture into autoclaved centrifuge bottles.
 - 2.3.2. Talent resuspending the bacterial pellet in a reduced volume of medium.
 - 2.3.3. Talent adding concentrated bacterial culture to the NGM plates and keeping it aside for incubation.
- 2.4. Using a sterile pipette tip, cut out a small chunk of agar containing approximately 1000 worms from a starved maintenance plate and place it onto a seeded NGM plate [1].
 - 2.4.1. Talent cutting out a chunk of agar from a maintenance plate using a pipette tip and transferring the agar chunk onto a seeded NGM plate.
- 2.5. After placing the new plate in a plastic container, incubate at 20 degrees Celsius for 3 days until the worms are gravid and laying eggs, while maintaining a sufficient bacterial lawn and preventing starvation [1].
 - 2.5.1. Talent placing the plastic container containing the seeded plate into an incubator.

3. Preparation of Experimental Plates

Demonstrator: Andreana Nourie, Garrick Tam

Notes: Emailed the authors to confirm the demonstrator. Will update upon their response.

- 3.1. To prepare experimental NGM plates for RNA interference or RNAi (*R-N-A-I*) by feeding, prepare 1 liter of NGM as previously demonstrated [1]. Using aseptic technique, add 1 milliliter of 1 molar Isopropyl beta-D-1-thiogalactopyranoside or IPTG (*I-P-T-G*) and 1 milliliter of 50 milligrams per milliliter ampicillin to the bottle [2]. Pour approximately 5 milliliters of medium into each 35-millimeter plate [3].
 - 3.1.1. Talent preparing NGM on the laboratory bench.
 - 3.1.2. CLOSE UP: Talent aseptically adding IPTG and ampicillin into the bottle.
 - 3.1.3. Talent pouring the medium into 35 millimeter plates.
- 3.2. Select the required number of *E. coli* HT115 (*H-T-one-fifteen*) RNAi feeding clones carrying L4440 (*L forty-four-forty*) plasmids targeting *C. elegans* genes of interest, together with the empty vector control [1]. Using an inoculating loop, pick a single colony and transfer it into 1.5 milliliters of LB medium containing 50 micrograms per

milliliter ampicillin [2]. Incubate for 18 hours at 37 degrees Celsius on an orbital shaker at 200 rpm [3].

3.2.1. Shot of labeled bacterial clone plates and empty vector control plates on the bench. Videographer's Notes: C035 is labeled at 3.2.1 CU. It's actually 3.2.2 CU.

3.2.2. CLOSE UP: Talent transferring a single bacterial colony into LB medium using an inoculating loop.

3.2.3. Talent placing the culture tubes onto an orbital shaker.

3.3. Seed 35-millimeter NGM plates containing ampicillin and IPTG with 100 microliters of overnight *E. coli* HT115 culture, preparing two technical replicates per condition, and incubate the plates overnight at room temperature [1]. Store an equal number of unseeded plates at 4 degrees Celsius for later egg transfer [2].

3.3.1. Talent pipetting *E. coli* HT115 culture onto 35-millimeter NGM plates and keeping it aside for incubation.

3.3.2. Talent storing the unseeded plates in the refrigerator. Videographer's Notes: C040 is labeled as 3.3.2-Talent storing the unseeded plates in the fridge. C073 is labeled at 3.3.2 is a duplicate. Author prefers to use C040

3.4. To prepare experimental NGM plates with a nutritional or drug supplement, prepare 1.5x NGM as previously demonstrated, but add 642 milliliters of distilled water instead of 975 milliliters [1]. Place the prepared medium in a 55 degrees Celsius water bath [2].

3.4.1. Talent adding distilled water into the concentrated Nematode Growth Medium. Videographer's Notes: C087 is labeled as 3.4.1- Labeled correctly, C084 is labeled as 3.4.1-Garrick with orange lid. This is actually 4.1.3.

3.4.2. Talent placing the prepared medium bottle into a water bath.

3.5. To prepare a water-based stock of the supplement at 3x the target concentration, calculate the required stock volume by multiplying the number of experimental plates by 1.666 milliliters [1]. Using a syringe filter, filter-sterilize the stock solution [2] and warm it to 55 degrees Celsius in a water bath [3].

3.5.1. Talent calculating the stock volume in a lab note.

3.5.2. Talent filter-sterilizing the supplement stock using a syringe filter.

3.5.3. Talent placing the stock solution into a water bath.

3.6. In a 50-milliliter conical tube, mix 2 parts of 1.5x NGM with 1 part supplement stock [1]. For the no-supplement control, mix 2 parts 1.5x NGM with 1 part hot sterile water, and

gently invert the tube 10 times to mix [2]. Using aseptic technique, quickly pour approximately 5 milliliters of medium into each 35-millimeter plate [3].

3.6.1. CLOSE UP: Talent mixing NGM and supplement stock in a 50-milliliter conical tube.

3.6.2. CLOSE UP: Talent mixing NGM with hot sterile water and inverting the conical tube multiple times to mix.

3.6.3. Talent pouring the prepared medium into 35 mm plates near a Bunsen burner.

3.7. After aseptically adding 10 milliliters of LB medium into a 50-milliliter Erlenmeyer flask, inoculate the medium with a colony of *E. coli* OP50 [1]. Incubate overnight at 37 degrees Celsius on an orbital shaker at 200 rpm [2].

3.7.1. CLOSE UP: Talent inoculating the LB medium in the 50 milliliter Erlenmeyer flask with an *E. coli* OP50 colony.

3.7.2. Talent placing the flask onto an orbital shaker.

3.8. Seed 35-millimeter NGM plates with 100 microliters of overnight *Escherichia coli* OP50 culture, preparing two technical replicates per condition, and incubate the plates overnight at room temperature [1].

3.8.1. Talent pipetting *E. coli* OP50 culture onto 35 mm NGM plates and keeping the plates aside for incubation.

4. Obtaining a Synchronized Population of L1 Larvae

Demonstrator: Garrick Tam

4.1. Gather the required reagents [1] to prepare alkaline bleach solution and M9 (*M-nine*) buffer [2]. After autoclaving at 121 degrees Celsius for 20 minutes and allowing the solution to cool, aseptically add 1 milliliter of one molar magnesium sulfate [3].

4.1.1. Talent arranging the reagents on the workbench.

4.1.2. TEXT ON A PLAIN BACKGROUND:

Alkaline Bleach Solution:

Sterile water - 7 mL

5% sodium hypochlorite solution - 2 mL

5 N NaOH -1 mL

M9 Buffer

KH₂PO₄ - 3 g

Na₂HPO₄ - 6 g

NaCl - 5 g

Distilled water -1 L

Adjust pH to 6.5

- 4.1.3. Talent aseptically adding 1 mL of 1 M magnesium sulfate to the cooled M9 buffer. Videographer's Notes: C084 is labeled as 3.4.1-Garrick with orange lid. This is actually 4.1.3.
- 4.2. Wash off the gravid adult worms from the plate with M9 buffer and transfer them into a 15-milliliter conical tube [1]. After centrifuging for 2 minutes at 350 g and aspirating the supernatant, fill the tube with fresh M9 buffer and repeat the wash 2 times [2].
 - 4.2.1. Talent adding M9 buffer to the plate to wash off the worms and transferring the suspension into a 15 mL conical tube.
 - 4.2.2. Talent resuspending the worm pellet in fresh M9 buffer and removing the supernatant
- 4.3. Add 2 milliliters of M9 buffer and 2 milliliters of alkaline bleach solution to the worm pellet and mix by vortexing and vigorous shaking [1]. Under the microscope, check periodically until nearly all worms are dissolved [2].
 - 4.3.1. Talent adding M9 buffer and alkaline bleach solution into the tube containing the worm pellet, vortexing, and vigorously shaking the tube. Videographer's Notes: 2964 is 4.3.1.B Worms in mid-dissolve, where some worms are dissolved and some embryos are present
 - 4.3.2. SCOPE: Show worms dissolving in the solution with only eggs remaining visible. Videographer's Notes: 2963 is labeled 4.3.2, 2965 is 4.3.2
- 4.4. After filling the tube with M9 buffer, centrifuge for 2 minutes at 350 g [1]. Invert the tube to remove the supernatant and wash the pellet 4 times with M9 buffer, ensuring the first wash is performed immediately after worm lysis to prevent overbleaching [2]. Then incubate the pellet in 8 milliliters of M9 buffer on a rocker for 18 to 20 hours until the eggs hatch [3].
 - 4.4.1. Talent placing the tube into the centrifuge.
 - 4.4.2. Talent removing the supernatant by inverting the tube, adding M9 buffer to the pellet, and again removing the supernatant.
 - 4.4.3. Talent placing the tube onto a rocker for incubation.

5. Embryo Transfer and Embryonic Lethality Analysis

Demonstrator: Andreana Nourie, Garrick Tam

Notes: Emailed the authors to confirm the demonstrator. Will update upon their response

- 5.1. Place five 3 microliter drops of worm suspension onto a glass slide [1]. Under a microscope, count the number of hatched L1 (*L-one*) larvae and divide the total count by 15 to determine the concentration per microliter [2].
 - 5.1.1. Talent pipetting multiple drops of worm suspension onto a glass slide.
 - 5.1.2. SCOPE: Show hatched L1 larvae under the microscope as they are counted.
Videographer's Notes: 5.1.2.SCOPE, 5.1.2.SCOPE-2. – No audio label, 5.1.2.SCOPE-3. – No audio label.
- 5.2. Plate 30 L1 larvae onto each seeded 35-millimeter plate [1]. After incubating the L1 larvae at 20 degrees Celsius for 72 hours, if developmental delay occurs under the experimental conditions, extend the incubation period until the worms become gravid and begin laying eggs [2].
 - 5.2.1. CLOSE UP: Talent transferring L1 larvae onto seeded 35-millimeter plates.
 - 5.2.2. SCOPE: Show gravid worms and eggs on the plate after incubation.
Videographer's Notes: 5.2.2. SCOPE, 5.2.2. SCOPE-2 - This has an audio label as 5.4.1. Disregard the audio label, 5.2.2.SCOPE-3, 5.2.2.SCOPE-4 – No audio label, 5.2.2.SCOPE-5
- 5.3. Remove the unseeded 35-millimeter plates from the refrigerator and allow them to reach room temperature [1].
 - 5.3.1. Talent removing unseeded 35-millimeter plates from a refrigerator and placing them on the laboratory bench.
- 5.4. Using a microscope, examine the worm plates and confirm that they contain more than 100 embryos with no hatched L1 larvae present [1].
 - 5.4.1. SCOPE: Show embryos on the worm plate with no visible hatched L1 larvae.
Videographer's Notes: 5.4.1.SCOPE – no audio label, 5.4.1.SCOPE-2
- 5.5. After carefully filling the plate with M9 buffer, using a vacuum line, aspirate the liquid to remove the maternal population and ensure that no adult worms remain on the plate [1].
 - 5.5.1. CLOSE UP: Talent aspirating liquid from the plate using a vacuum line.
Videographer's Notes: Labeled as 5.5.1 but are actually scopes shots of the talent aspirating liquid from the plate using a vacuum line to remove the maternal population. 5.5.1.SCOPE – Audio label 5.1.1, 5.5.1.SCOPE-2 – Audio label 5.1.1, 5.5.1.SCOPE-3 – No audio label
- 5.6. Once the micropipette tip is filled with 200 microliters of M9 buffer, pipette up and down forcefully to detach embryos from the bacterial lawn [1]. While viewing through the microscope, guide the pipette tip toward embryo-dense areas [2].
 - 5.6.1. CLOSE UP: Talent pipetting up and down on the bacterial lawn to detach

embryos.

5.6.2. SCOPE: Show the pipette tip approaching embryo-dense regions on the plate.

Videographer's Notes: 5.6.2.SCOPE

5.7. Take an unseeded plate and pipette a drop of embryo suspension into the center [1]. After examining the droplet under the microscope, add more of the embryo suspension until the droplet contains 100 to 300 embryos, and ensure that no worms from the parental population are transferred with the embryos [2].

5.7.1. CLOSE UP: Talent pipetting a drop of embryo suspension into the center of an unseeded plate.

5.7.2. SCOPE: Show additional embryo suspension being added to the droplet on the plate. Videographer's Notes: 5.7.2.SCOPE – Audio label is 5.6.2. Disregard the audio label

5.8. Once the liquid is dried on the transfer plate, incubate the plates at 20 degrees Celsius for at least 24 hours [1].

5.8.1. Talent placing the transfer plates into an incubator set to 20 degrees Celsius.

5.9. After drawing a 4-millimeter by 4-millimeter grid on the lid of a 35-millimeter Petri dish, place the lid beneath the plate and use the grid as a guide [1]. Using a microscope and a handheld click counter, count the number of L1 larvae and unhatched embryos [2].

5.9.1. Talent positioning the gridded lid beneath the plate.

5.9.2. SCOPE: Show L1 larvae and unhatched embryos being counted under the microscope while the handheld click counter is used. Videographer's Notes: 5.9.2.SCOPE

5.10. Sum the numbers of unhatched embryos and hatched larvae across the technical replicates to ensure appropriate weighting based on progeny count [1].

5.10.1. SCREEN: 71559_screenshot_1.mov: 00:00- 00:12 *Video editor: Emphasize the columns, embryos, and worms*

5.11. Calculate the embryonic lethality rate for each biological replicate by dividing the number of embryos by the total number of worms and embryos, and multiplying the value by 100 to express the embryonic lethality rate as a percentage [1].

5.11.1. SCREEN: 71559_screenshot_2.mov: 00:11-00:32, 00:57-01:01

5.12. Repeat the entire experiment at least 3 times using 2 technical replicate plates per condition with a minimum of 100 embryos per replicate, while performing statistical analysis of embryonic lethality rates [1].

5.12.1. SCREEN: 71559_screenshot_3.mov: 00:00-00:12. *Video editor: In column A, circle and emphasize the rows 4 to 9.*

Results

6. Results

- 6.1. In the embryonic lethality assay, T04G9.4 (*T-zero-four-G-nine-point-four*) knockdown produced nearly fully penetrant embryonic lethality in both RNAi-hypersensitive [1] and wild-type animals [2].
 - 6.1.1. LAB MEDIA: Figure 2A. *Video editor: Emphasize the bar labeled T04G9.4 in the rrf-3(pk1426) panel, along with the black dots*
 - 6.1.2. LAB MEDIA: Figure 2B. *Video editor: Emphasize the bar labeled T04G9.4 in the N2 (wild type) panel, along with the black dots*
- 6.2. Phenotypes approaching 0% lethality, such as vector [1], or approaching 100% lethality, such as T04G9.4 and K08E3.5 (*K-zero-eight-E-three-point-five*), were less variable [2].
 - 6.2.1. LAB MEDIA: Figure 2A,B. *Video editor: Emphasize the vector bars in both rrf-3(pk1426) and N2 (wild type) panel, along with the black dots*
 - 6.2.2. LAB MEDIA: Figure 2A,B. *Video editor: Emphasize the K08E3.5 and T04G9.4 bar in rrf-3(pk1426) panel and the T04G9.4 bar in N2 (wild type) panel, along with the black dots*
- 6.3. RNAi knockdowns with intermediate penetrance, such as B0272.3 (*B-zero-two-seventy-two-point-three*) [1] and fat-6 (*fat-six*), displayed higher variance [2].
 - 6.3.1. LAB MEDIA: Figure 2B. *Video editor: Emphasize the B0272.3 bar along with the black dots*
 - 6.3.2. LAB MEDIA: Figure 2B. *Video editor: Emphasize the fat-6 bar along with the black dots*
- 6.4. The results confirmed previously reported embryonic lethal phenotypes and revealed a broad range in the severity of embryonic lethality [1].
 - 6.4.1. LAB MEDIA: Figure 2A,B.

Pronunciation guides:

1. Nematode
Pronunciation link: <https://www.merriam-webster.com/dictionary/nematode>
IPA: /'ni:mə,təʊd/
Phonetic Spelling: nee·muh·tohd
2. Aseptically
Pronunciation link: <https://www.merriam-webster.com/dictionary/aseptic>
IPA: /eɪ'sep.tɪk.li/
Phonetic Spelling: ay·SEP·tik·lee
3. Autoclave
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>
IPA: /'ɔ:təʊ,kleɪv/
Phonetic Spelling: aw·toh·klayv
4. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sen.trə,fju:dʒ/
Phonetic Spelling: sen·truh·fyooj
5. Cholesterol
Pronunciation link: <https://www.merriam-webster.com/dictionary/cholesterol>
IPA: /kə'les.tə,rɔ:l/
Phonetic Spelling: kuh·LES·tuh·rawl
6. Hypochlorite
Pronunciation link: <https://www.merriam-webster.com/dictionary/hypochlorite>
IPA: /,haɪ.pəʊ'klɔ:r,aɪt/
Phonetic Spelling: hy·poh·KLOR·ite
7. Escherichia
Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia>
IPA: /,eʃə'ɪkiə/
Phonetic Spelling: esh·uh·RIK·ee·uh
8. coli
Pronunciation link: <https://www.merriam-webster.com/dictionary/E.%20coli>
IPA: /'kəʊlaɪ/
Phonetic Spelling: KOH·lye
9. Micropipette
Pronunciation link: <https://www.merriam-webster.com/dictionary/micropipette>
IPA: /,maɪkroʊpaɪ'pet/
Phonetic Spelling: MY·kroh·pie·PET
10. Microliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊ.li:tər/
Phonetic Spelling: MY·kroh·lee·ter
11. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>

IPA: /'maɪkrəˌskoʊp/

Phonetic Spelling: MY·kruh·skohp

12. Phosphate

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

IPA: /'fɑːsˌfeɪt/

Phonetic Spelling: FAHS·fayt

13. Magnesium

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

IPA: /mæg'niːziəm/

Phonetic Spelling: mag·NEE·zee·um

14. Sulfate

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

IPA: /'sʌlˌfeɪt/

Phonetic Spelling: SUL·fayt

15. Pipette

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

IPA: /paɪ'pet/

Phonetic Spelling: pie·PET

16. Incubate

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

IPA: /'ɪŋkjəˌbeɪt/

Phonetic Spelling: ING·kyuh·bayt