

Submission ID: 70434

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21482418>

Title: Small Scale Dissection and Antibody Staining of Eye-Antennal and Wing Imaginal Discs from *Drosophila melanogaster*

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? **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. **YES**

Current Protocol Length

Number of Steps: 15

Number of Shots: 48 (plus 8 added SCOPE shots)

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

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

- 1.1. **Olivia Monet:** Our lab primarily uses *Drosophila* as a model organism to study ribosomopathies and cell competition.

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

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Olivia Monet:** This protocol uses smaller reagent volumes than existing methods and devotes individual attention to each sample.

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. **Nicholas Baker:** This protocol is primarily used for immunohistochemistry and confocal detection of proteins but can be adapted for other kinds of labeling.

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. **Olivia Monet:** A key challenge of this protocol is that it requires additional time and careful handling, as each specimen is processed individually.

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. **Olivia Monet:** This protocol was developed for *Drosophila* imaginal discs and could be applied to any other comparably sized tissue.

- 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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What problem in your field of research does your JoVE article address?

- 1.1. **Olivia Monet, Junior Specialist at University of California, Irvine and Nicholas Baker, Professor at University of California, Irvine:** (authors will present their testimonial statements live)

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

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

- 1.2. **Olivia Monet, Junior Specialist at University of California, Irvine and Nicholas Baker, Professor at University of California, Irvine:** (authors will present their testimonial statements live)

1.2.1. 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: LAB MEDIA/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Kathyayani Sridharan) for queries regarding lab media.

2. Dissection and Fixation of *Drosophila* Eye-Antennal Discs and Wing Imaginal Discs

Demonstrator: Olivia Monet

2.1. To begin, obtain a tungsten wire to prepare the tissue transfer tool [1-TXT]. Use pliers to cut it into approximately 2.5-centimeter piece [2]. Use forceps to shape one end of the wire into a hook [3] or twist into a loop [4] and insert the other end of the wire into a needle holder [5].

2.1.1. WIDE: Talent bringing a tungsten wire and placing it on a workbench. **TXT: Diameter: 0.005 in**

2.1.2. Talent cutting a tungsten wire with pliers.

2.1.3. LAB MEDIA: 70434-2-1-3. 00:00:00 – 00:00:20

2.1.4. LAB MEDIA: 70434-2-1-4. 00:00:00 – 00:00:44

2.1.5. LAB MEDIA: 70434-2-1-5. 00:00:00 – 00:00:20

2.2. Transfer the larvae into Petri dish containing 7.5 milliliters of 0.1 molar sodium phosphate buffer at pH 7.2 [1]. Gently swirl the larvae in the buffer solution to rinse off any fly media or debris.[2]. Transfer the larvae to a petri dish containing fresh buffer [3].

2.2.1. Talent transferring the larvae into 60 mm dish with buffer.

2.2.2. Talent gently swirling the dish to remove debris from larvae.

2.2.3. Talent transferring the larvae to a new dish with buffer.

2.3. Observe the larva under a dissecting microscope [1] and use forceps to secure the larva at approximately fifty-percent body length [2] Use a second set of fine forceps to grasp the mouth hooks and pull steadily to isolate the eye-antennal discs.[3]. If the brain remains attached to the eye-antennal discs, it can be removed now or at a later stage [4]. **Note: The numbers and narration are adjusted for the added shot.**

2.3.1. Talent placing and observing the petri dish under a dissecting microscope.

2.3.2. LAB MEDIA: 70434-2-3-2&3a. 00:00:00 – 00:00:03

Note: SCOPE shots 2.3.2 and 2.3.3 were filmed as a single video; separate timestamps are shown for separate actions. Authors added another version

with different outcome as additional SCOPE shot. The corresponding lab media, timestamps and narration is added for the new shot.

2.3.3. LAB MEDIA: 70434-2-3-2&3a. 00:00:04 – 00:00:22

Added shot: LAB MEDIA: 70434-2-3-2&3b. 00:00:12 – 00:00:36

2.4. To obtain the wing imaginal discs, secure the larval mouthparts with fine forceps [1], then use microdissection scissors to bisect the larva through the upper third of the body [2]. Using a second pair of forceps, spread the cut end [3] and push the head and mouthparts through similar to inverting a sock [4].

2.4.1. LAB MEDIA: 70434-2-4-1&2&3&4. 00:00:00 – 00:00:10 **Note: SCOPE shots 2.4.1, 2.4.2, 2.4.3 and 2.4.4 were combined into a single video named 2.4.1&2&3&4; separate timestamps are shown for separate actions.**

2.4.2. LAB MEDIA: 70434-2-4-1&2&3&4. 00:00:11 – 00:00:16

2.4.3. LAB MEDIA: 70434-2-4-1&2&3&4. 00:00:17 – 00:00:22

2.4.4. LAB MEDIA: 70434-2-4-1&2&3&4. 00:00:23 – 00:00:31

2.5. Use the wire tool to transfer the dissected tissue into separate 35-millimeter Petri dishes each containing approximately three milliliters fixative solution consisting of 3.7 percent formaldehyde in 0.1 molar sodium phosphate [1]. After fifteen to twenty minutes at room temperature, transfer the dissected tissues into another Petri dish containing 3 milliliters of 0.1 molar sodium phosphate buffer at pH 7.2 for washing [2].

2.5.1. Talent transferring the dissected tissues into separate Petri dishes containing fixative.

2.5.2. Talent transferring the fixed tissues to separate Petri dishes containing sodium phosphate buffer.

3. Antibody Staining and Mounting of Eye-Antennal and Wing Imaginal Discs

3.1. Prepare NSG (*N-S-G*) antibody wash buffer [1] for blocking non-specific sites on the tissues [2]. Transfer the eye-antennal disc [3] and the wing imaginal disc tissues into Petri dishes containing 3 milliliters of NSG antibody wash buffer [4]. Incubate on ice for at least fifteen minutes [5]. **Note: The numbers and narration are adjusted for the added shot.**

3.1.1. Talent filter-sterilizing the NSG wash buffer.

3.1.2. TEXT ON A PLAIN BACKGROUND:

NSG Ab wash buffer:

0.1 M sodium phosphate buffer (pH 7.2)

0.1% saponin

5% Normal Goat Serum.

Filter-sterilize the stock and store at 4 °C

3.1.3. LAB MEDIA: 70434-3-1-3a. 00:00:00 – 00:00:16 **Note: SCOPE shot 3.1.3 was recorded as two videos. The specific LAB MEDIA, time stamps and corresponding narration are added.**

Added shot: LAB MEDIA: 70434-3-1-3b. 00:00:00 – 00:00:19

3.1.4. Talent placing the Petri dishes on ice.

- 3.2. Use fine forceps to clear extraneous tissue from the eye-antennal discs while retaining the mouth hooks to facilitate handling [1]. Similarly for wing discs, retain the haltere and leg discs for the ease of handling [2].
- 3.2.1. LAB MEDIA: 70434-3-2-1. 00:00:00 – 00:00:28
 - 3.2.2. LAB MEDIA: 70434-3-2-2. 00:00:00 – 00:00:21
- 3.3. In a sixty-well microwell minitray, add 13 microliters of primary antibody solution to six wells in the first column [1-TXT]. Use the hook transfer tool to move the dissected eye-antennal tissue [2] to the primary antibody solution [3]. Similarly, use the wire loop tool to transfer the wing imaginal discs [4] into the primary antibody solution [5]. Close the plate with the lid [6], seal with parafilm to limit evaporation [7] and incubate at four degrees Celsius overnight [8]. **Note: The numbers and narration are adjusted for the added shots.**
- 3.3.1. LAB MEDIA: 70434-3-3-1. 00:00:00 – 00:00:17 **TXT: Primary Ab: 1:100 mAb40-1a in NSG; Mix by pipetting only**
 - 3.3.2. LAB MEDIA: 70434-3-3-2a1. 00:00:00 – 00:00:10 **Note: SCOPE shot 3.3.2 was recorded as four videos. The specific LAB MEDIA, time stamps and corresponding narration are added.**
 - Added shot: LAB MEDIA: 70434-3-3-2a2. 00:00:00 – 00:00:13
 - Added shot: LAB MEDIA: 70434-3-3-2b1. 00:00:00 – 00:00:17
 - Added shot: LAB MEDIA: 70434-3-3-2b2. 00:00:00 – 00:00:04
 - 3.3.3. Talent closing the tray with lid. *Videographer: Obtain multiple reusable takes of 3.3.3-3.3.5 as these will be used later in the protocol*
 - 3.3.4. Talent sealing the tray with parafilm.
 - 3.3.5. Talent placing the tray on ice or in a refrigerator.
- 3.4. After removing the plate from incubator, add 13 microliters of NSG buffer to the subsequent three columns of wells [1]. Wash the tissue samples thrice by placing them in each well of NSG buffer successively [2].
- 3.4.1. LAB MEDIA: 70434-3-4-1. 00:00:00 – 00:00:44
 - 3.4.2. LAB MEDIA: 70434-3-4-2. 00:00:00 – 00:00:49
- ~~Videographer: Obtain multiple reusable takes of 3.4.1 and 3.4.2 as these will be used later in the protocol~~
- 3.5. Centrifuge the secondary antibody stock solution for sixty seconds before dilution, as antibody aggregates can bind nonspecifically to the tissue surface [1-TXT]. Prepare a 1 in 200 to 1 in 400 dilution of fluorescently conjugated secondary antibodies in NSG buffer [2] and mix by pipetting [3].
- 3.5.1. Talent centrifuging the secondary antibody stock. **TXT: Secondary Ab: Alexa Fluor 488 Donkey Anti-Mouse IgG**
 - 3.5.2. Talent diluting the secondary antibody in the NSG buffer.
 - 3.5.3. Talent mixing the antibody by pipetting.

- 3.6. Add 13 microliters of secondary antibody solution to the fifth column of wells [1]. Transfer the samples into the secondary antibody using the loop [2]. Close the plate with the lid, seal with parafilm and incubate on ice for four hours [3].
 - 3.6.1. LAB MEDIA: 70434-3-6-1&2. 00:00:00 – 00:00:18 **Note: SCOPE shots 3.6.1 and 3.6.2 are combined into single video. Separate time stamps are shown for separate actions.**
 - 3.6.2. LAB MEDIA: 70434-3-6-1&2. 00:00:19 – 00:00:46
 - 3.6.3. *Reuse 3.3.3; 3.3.4 and 3.3.5.*
- 3.7. Add 13 microliters of NSG buffer to the subsequent three columns of wells [1] for washing the tissues thrice by placing them in each well successively [2]. Then, add 13 microliters of 0.1 molar sodium phosphate buffer at pH 7.2 to the ninth well of the minitray [3] and transfer the tissue samples for the final wash [4]. **Note: The narration and numbers are adjusted for the added shots.**
 - 3.7.1. LAB MEDIA: 70434-3-7-1. 00:00:00 – 00:00:45 **Note: SCOPE shots 3.7.1 and 3.7.2 are split into two parts due to its length; the second parts are labeled as an "added shot" and corresponding narration is adjusted.**
Added shot: LAB MEDIA: 70434-3-7-1. 00:00:46 – 00:01:34
 - 3.7.2. LAB MEDIA: 70434-3-7-2. 00:00:00 – 00:00:10
Added shot: LAB MEDIA: 70434-3-7-2. 00:00:11 – 00:00:59
- 3.8. Now add the mounting media to the tenth column [1] of the minitray [2] and transfer the samples into it using the transfer tool [3].
 - 3.8.1. LAB MEDIA: 70434-3-8-3. 00:00:00 – 00:00:19 **Note: Please use the LAB MEDIA for this shot, because the step can be better demonstrated this way.**
 - 3.8.2. TEXT ON A PLAIN BACKGROUND
Mounting media:
2% n-propyl gallate in 75% glycerol
25% 0.1 M sodium phosphate buffer, pH 7.2
 - 3.8.3. LAB MEDIA: 70434-3-8-3. 00:00:20 – 00:01:03
- 3.9. Apply twenty microliters of mounting medium to a clean slide [1]. Transfer the washed tissues into the mountant droplet [2]. At this stage, remove the mouthparts from the eye-antennal discs for better visualization [3]. Use fine forceps to position the tissue outside the main drop and prevent drifting [4]. Gently lower a coverslip onto the mountant, taking care to avoid air bubbles [5-TXT]. **Note: The narration and numbers are adjusted for the added shot.**
 - 3.9.1. Talent adding mounting media to the glass slide.
 - 3.9.2. LAB MEDIA: 70434-3-9-2. 00:00:00 – 00:00:14
 - 3.9.3. LAB MEDIA: 70434-3-9-3. 00:00:00 – 00:00:58 **Note: SCOPE shot 3.9.3 is split into two parts due to its length; the second part is labeled as an "added shot" and corresponding narration is updated.**

Added shot: LAB MEDIA: 70434-3-9-3. 00:00:59 – 00:02:21

3.9.4. LAB MEDIA: 70434-3-9-4. 00:00:00 – 00:00:31 TXT: Coverslip: Thickness: #1.5; size: 22 × 40 mm

3.10. Allow the slide to settle for one hour in a cardboard holder [1], then seal the coverslip edges with nail polish without applying pressure [2]. Store the slides at four degrees Celsius, once the sealant has dried [3].

3.10.1. Talent placing the slide in cardboard holder.

3.10.2. Talent sealing the edges with nail polish.

3.10.3. Talent placing the slides in the refrigerator.

Results

4. Results:

- 4.1. In the present study, a third-instar eye-antennal imaginal disc was triple-labeled to detect the transcription factor Senseless [1], neural progenitor marker Elav (*el-uh-vee*) [2], and anti-GFP (*G-F-P*) labelled cell clones expressing RNAi (*R-N-A-eye*) for the Ocho (*OH-choh*) transcript [3].
 - 4.1.1. LAB MEDIA: Figure 5A *Video Editor: Please emphasize the blue region.*
 - 4.1.2. LAB MEDIA: Figure 5A *Video Editor: Please emphasize the red region.*
 - 4.1.3. LAB MEDIA: Figure 5A *Video Editor: Please emphasize the green region.*
- 4.2. Similarly, a third-instar wing imaginal disc is labeled with the TIE-DYE (*tai-die*) system, marking clonal lineages via anti-GFP [1], anti-beta-galactosidase [2], and endogenous RFP (*R-F-P*) [3].
 - 4.2.1. LAB MEDIA: Figure 5B *Video Editor: Please emphasize the green region.*
 - 4.2.2. LAB MEDIA: Figure 5B *Video Editor: Please emphasize the blue region.*
 - 4.2.3. LAB MEDIA: Figure 5B *Video Editor: Please emphasize the red region.*

Pronunciation Guide:

1. Dissection
Pronunciation link: <https://www.merriam-webster.com/dictionary/dissection>
IPA: /dɪ'sekʃən/
Phonetic Spelling: dih-sek-shuhn
2. Fixation
Pronunciation link: <https://www.merriam-webster.com/dictionary/fixation>
IPA: /fɪk'seɪʃən/
Phonetic Spelling: fik-say-shuhn
3. Drosophila
Pronunciation link: <https://www.merriam-webster.com/dictionary/Drosophila>
IPA: /drə'säfələ/
Phonetic Spelling: druh-saa-fuh-luh
4. Antennal
Pronunciation link: <https://www.merriam-webster.com/dictionary/antennal>
IPA: /æn'tenəl/
Phonetic Spelling: an-ten-uhl
5. Imaginal
Pronunciation link: <https://www.merriam-webster.com/dictionary/imaginal>
IPA: /ɪ'mædʒənəl/
Phonetic Spelling: ih-maj-uh-nuhl
6. Demonstrator
Pronunciation link: <https://www.merriam-webster.com/dictionary/demonstrator>

IPA: /'demənˌstreɪtər/

Phonetic Spelling: deh·muhn·stray·tuhr

7. Tungsten

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

IPA: /'təŋstən/

Phonetic Spelling: tung·stuhn

8. Forceps

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

IPA: /'fɔːsɪps/

Phonetic Spelling: for·seps

9. Sodium

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

IPA: /'soʊdiəm/

Phonetic Spelling: soh·dee·uhm

10. Phosphate

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

IPA: /'fɑːsˌfeɪt/

Phonetic Spelling: faas·fayt

11. Larvae

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

IPA: /'lɑːrˌviː/

Phonetic Spelling: laar·vee

12. Debris

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

IPA: /də'brɪː/

Phonetic Spelling: duh·bree

13. Microscope

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

IPA: /'maɪkrəˌskoʊp/

Phonetic Spelling: my·kruh·skohp

14. Isolate

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

IPA: /'aɪsəˌleɪt/

Phonetic Spelling: eye·suh·layt

15. Microdissection

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

IPA: /ˌmaɪkrəʊdɪ'sekʃən/

Phonetic Spelling: my·kroh·dih·sek·shuhn

16. Bisect

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

IPA: /baɪ'sekt/

Phonetic Spelling: by·sekt

17. Formaldehyde
Pronunciation link: <https://www.merriam-webster.com/dictionary/formaldehyde>
IPA: /fər'mældəˌhaɪd/
Phonetic Spelling: fur-mal-duh-hyde
18. Antibody
Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>
IPA: /'ænˌtɪˌbɑːdi/
Phonetic Spelling: an-tih-baa-dee
19. Staining
Pronunciation link: <https://www.merriam-webster.com/dictionary/staining>
IPA: /'steɪnɪŋ/
Phonetic Spelling: stay-ning
20. Mounting
Pronunciation link: <https://www.merriam-webster.com/dictionary/mounting>
IPA: /'maʊntɪŋ/
Phonetic Spelling: moun-ting
21. Saponin
Pronunciation link: <https://www.merriam-webster.com/dictionary/saponin>
IPA: /'sæpənɪn/
Phonetic Spelling: sap-uh-nin
22. Serum
Pronunciation link: <https://www.merriam-webster.com/dictionary/serum>
IPA: /'sɪrəm/
Phonetic Spelling: seer-uhm
23. Sterilize
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilize>
IPA: /'sterəˌlaɪz/
Phonetic Spelling: steh-ruh-lyze
24. Haltere
Pronunciation link: <https://www.howtopronounce.com/haltere>
IPA: /hæl'tɪr.iː/
Phonetic Spelling: hal-teer-ee
25. Microwell
Pronunciation link: <https://www.merriam-webster.com/dictionary/microwell>
IPA: /'maɪkroʊˌwel/
Phonetic Spelling: my-kroh-wel
26. Minitray
Pronunciation link: No confirmed link found
IPA: /'mɪniˌtreɪ/
Phonetic Spelling: mih-nee-tray
27. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>

IPA: /'ɪŋkjəˌbeɪt/

Phonetic Spelling: ing·kyuh·bayt

28. Centrifuge

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

IPA: /'sentərəˌfjuːdʒ/

Phonetic Spelling: sen·truh·fyooj

29. Nonspecifically

Pronunciation link: No confirmed link found

IPA: /ˌnɒnspeɪˈsɪfɪkli/

Phonetic Spelling: non·spuh·sif·ik·lee

30. Fluorescently

Pronunciation link: No confirmed link found

IPA: /flɒ'resəntli/

Phonetic Spelling: floo·reh·suhnt·lee

31. Conjugated

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

IPA: /'kɔːndʒəˌgeɪtɪd/

Phonetic Spelling: kaan·juh·gay·tuhd

32. Alexa

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

IPA: /əˈleksə/

Phonetic Spelling: uh·lek·suh

33. Glycerol

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

IPA: /'glɪsəˌrɔːl/

Phonetic Spelling: glis·uh·rawl

34. Mountant

Pronunciation link: No confirmed link found

IPA: /'məʊntənt/

Phonetic Spelling: moun·tuhnt

35. Coverslip

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

IPA: /'kʌvərˌslɪp/

Phonetic Spelling: kuh·vuhr·slip

36. Visualization

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

IPA: /ˌvɪʒʊələˈzeɪʃən/

Phonetic Spelling: vizh·oo·uhl·uh·zay·shuhn

37. Parafilm

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

IPA: /'pærəˌfɪlm/

Phonetic Spelling: pair·uh·film

38. Propyl

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

IPA: /'proʊpəl/

Phonetic Spelling: proh·puhl

39. Fluor

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

IPA: /'flʊr/

Phonetic Spelling: floor

40. Refrigerate

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

IPA: /rɪ'frɪdʒəˌreɪt/

Phonetic Spelling: ruh·frij·uh·rayt