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Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21531523>

**Title: A Standardized Protocol for Inducing Stress-Induced Premature Senescence in Primary Human Melanocytes Using Tert-butyl Hydroperoxide**

**Authors and Affiliations:**

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<sup>3</sup>European Innovation Center

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| Maria Cavinato        | maria.cavinato-nascimento@uibk.ac.at |

## 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 shots:

2.1.2.

2.4.2.

4.8.1

4.8.2.

**NOTE: These were filmed by the videographer**

**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: 27

Number of Shots: 41

# Interviews

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

**Authors:** Answers are slightly edited in accordance with journal guidelines. Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

## INTRODUCTION:

**Videographer's NOTE:** I shot the Interviews in 4K ProRes and the protocol in 1080 Rec.709. I picked 25 fps because 30 fps would lead to light flickering in Europe. The shots with the scope adapter were captured with an Iphone 15 also in ProRes Apple Log and a LUT is also included.

**1\_2\_1** INTERVIEW has the wrong slate number

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

- 1.1. **Maria Cavinato:** Our research focuses on investigating the senescent phenotype of melanocytes and its contribution to extrinsic skin aging.
  - 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. **Maria Cavinato:** Established senescence models use fibroblasts and fail to capture melanocyte-specific biology. This protocol induces tBHP-mediated oxidative stress in primary human melanocytes.
  - 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. **Maria Cavinato:** This model allows researchers to study how senescent melanocytes affect pigmentation and communicate with surrounding cells via SASP inflammatory factors.
  - 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. **Maria Cavinato**: The challenge in this protocol is precise timing. tBHP must be applied twice daily with at least a four-hour interval.

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. **Maria Cavinato**: Following this protocol, senescence can be characterized using SA- $\beta$ -galactosidase staining, immunofluorescence, RT-qPCR, and Western blotting of established senescence markers.

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.***

**Ethics Title Card**

This study utilized commercially obtained de-identified primary human neonatal melanocytes collected with informed donor consent in compliance with established ethical guidelines

# Protocol

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

## 2. Seeding Melanocytes

**Demonstrator:** Amina Hassan

- 2.1. To begin, gather the required reagents [1]. After thawing the melanocytes for 3 days, ensure that the cells reach 80 to 90 percent confluency on the seeding day [2].

- 2.1.1. WIDE: Talent waking into the room with a tray of reagents. ***Videographer:** Please obtain CU shot as well for this shot.*

**Videographer's NOTE:** (1\_1\_2)'2\_1\_1\_Protocol\_additional' is not fully approved by the author. She didn't like herself from the low angle so only the spraying part of the fluids can be used as a close up if needed

**Videographer's NOTE:** 2\_1\_1\_1\_Protocol and 2\_1\_1\_2\_Protocol were added to show some details of the preparation

- 2.1.2. SCOPE: Show adherent melanocytes at 80 to 90 percent confluency
- 2.2. Aspirate the medium from the flask [1]. Wash the cells twice with DPBS (*D-P-B-S*) [2].
- 2.2.1. Talent aspirating the medium from the culture flask.
- 2.2.2. Talent adding DPBS to the flask.
- 2.3. Add 2 milliliters of Trypsin-EDTA (*E-D-T-A*) solution to detach the cells [1]. Place the flask in the incubator for 5 minutes at 37 degrees Celsius with 5 percent carbon dioxide [2].
- 2.3.1. Talent adding Trypsin-EDTA solution into the culture flask.
- 2.3.2. Talent placing the flask into the incubator.
- 2.4. Gently tap the flask [1] and under the microscope observe the cells to confirm complete detachment [2].
- 2.4.1. Talent gently tapping the culture flask.
- 2.4.2. SCOPE: Show detached cells lifting from the flask surface under the microscope.
- 2.5. Add 1.5 times the volume of trypsin inhibitor to stop the reaction [1].
- 2.5.1. Talent adding trypsin inhibitor into the flask containing detached cells.
- 2.6. Transfer the cell suspension to a 15 milliliter conical tube [1]. Dilute the suspension with DPBS to a final volume of 10 milliliters [2].
- 2.6.1. Talent transferring the cell suspension into a 15 milliliter conical tube.

- 2.6.2. Talent adding DPBS into the conical tube containing cell suspension.
- 2.7. Centrifuge the cell suspension at a relative centrifugal force of 100 for 5 minutes [1].
  - 2.7.1. Talent placing the conical tube into the centrifuge.  
**Videographer's NOTE: '2\_7\_1\_Protocol\_wrong\_slate' has the wrong slate number (3\_7\_1)**
- 2.8. Aspirate the supernatant [1] and resuspend the cell pellet in medium to the desired volume [2].
  - 2.8.1. Talent aspirating the supernatant from the conical tube.
  - 2.8.2. Talent resuspending the cell pellet in fresh medium.
- 2.9. Seed 6 lakh cells per 10 centimeter dish for tert-butyl hydroperoxide or tBHP (*T-B-H-P*) treated plates [1]. Seed 4 lakh cells per plate for the control plates [2].
  - 2.9.1. Talent seeding cells into a 10 centimeter dish labeled tBHP.  
**Videographer's NOTE: Shot 2\_9\_1\_and\_2\_9\_2\_Protocol were combined into one'**
  - 2.9.2. Talent seeding cells into a plate labeled control.

### **3. Tert-Butyl Hydroperoxide (tBHP) Treatment**

- 3.1. Prepare a 10 millimolar working solution from the tBHP stock using DPBS or media [1].
  - 3.1.1. Talent adding tBHP solution in a tube containing DPBS or media.
- 3.2. Add 100 micromolar tBHP to the cells for 1 hour per treatment [1].
  - 3.2.1. Talent adding tBHP solution into the culture plate.  
**Videographer's NOTE: 3\_2\_1\_and\_3\_3\_1\_Protocol\_\_wrong\_slate  
Shot 3\_2\_1 and 3\_3\_1 were combined into one and it has the wrong slate number (3\_1\_1 - was used twice)**
- 3.3. Move the plate back and forth to distribute the tBHP evenly throughout the medium [1].
  - 3.3.1. Talent gently moving the plate back and forth.
- 3.4. Place the cells in the incubator for precisely 1 hour at 37 degrees Celsius with 5 percent carbon dioxide [1].
  - 3.4.1. Talent placing the culture plate into the incubator.
- 3.5. Aspirate the medium to terminate the tBHP treatment [1].
  - 3.5.1. Talent aspirating the treatment medium from the culture plate.

- 3.6. Wash the plates twice with pre-heated DPBS at 37 degrees Celsius, protecting them from light [1].
  - 3.6.1. Talent adding pre-heated DPBS to the plates.  
**Videographer's NOTE:** 3\_6\_1\_1\_Protocol was added because the experiment should be protected from light before going into the incubator
- 3.7. Add the appropriate volume of fresh medium to the plates [1].
  - 3.7.1. Talent adding fresh medium into the culture plates.
- 3.8. Place the plates in the incubator and allow the cells to recover for at least 4 hours before the next treatment [1].
  - 3.8.1. Talent placing the culture plates back into the incubator.
- 3.9. Repeat the treatment procedure later on the same day for the second treatment [1]. Continue the treatment regimen and maintain the cells in culture until Day 9 to assess senescence markers [2].
  - 3.9.1. Talent adding t-BHP to the previously treated culture plates.
  - 3.9.2. Shot of labeled culture plates in the incubator.

#### **4. Growth Curve Analysis and Senescence Assessment**

**Demonstrator:** Lena Guerrero-Navarro

- 4.1. Count the tBHP-treated cells and control cells [1].
  - 4.1.1. SCREEN: Show the cells on the Casy counter computer  
**Videographer's NOTE:** Shot 4\_1\_1 and Shot 4\_3\_1 display a cell counting machine because they use a different machine for counting cells which doesn't show the data as described
- 4.2. Reseed them at the same initial density or if fewer than 6 lakh cells are obtained from the tBHP-treated plates, reseed all collected cells [1].
  - 4.2.1. Talent reseeding cells into fresh culture plates.
- 4.3. Count the cells on Days 4, 9, and 15 of culture [1].
  - 4.3.1. SCOPE: Show melanocytes in the counting chamber.  
**Videographer's NOTE:** Shot 4\_1\_1 and Shot 4\_3\_1 display a cell counting machine because they use a different machine for counting cells which doesn't show the data as described
- 4.4. On Day 9, wash the cells twice with PBS [1]. Fix the cells using 2 percent formaldehyde and 0.4 percent glutaraldehyde [2].

- 4.4.1. Talent adding PBS to the culture plate.
- 4.4.2. Talent adding the fixation solution onto the cells.
- 4.5. Wash the fixed cells twice with PBS [1].
  - 4.5.1. Talent adding PBS to the cells.
- 4.6. Incubate the fixed cells in a staining solution at pH 6 [1] for 18 hours at 37 degrees Celsius in the absence of carbon dioxide [2].
  - 4.6.1. Talent adding the staining solution onto the fixed cells.
  - 4.6.2. Talent placing the stained culture plate into the incubator.
- 4.7. Wash the cells twice with PBS [1].
  - 4.7.1. Talent washing the stained cells with PBS.
- 4.8. Using light microscopy, image and count the number of blue-stained cells [1]. Divide it by the total number of cells within the same field [2]. Express the results as the percentage of positive cells [3].
  - 4.8.1. SCOPE: Show blue stained cells under light microscopy.
  - 4.8.2. SCOPE: Show the total cell population within the microscopic field.
  - 4.8.3. Show a printed data sheet showing the final percentage of positive cells.
- 4.9. Perform immunofluorescence staining [1], RT-qPCR [2], and Western blotting to further assess senescence markers in tBHP-treated melanocytes [3].
  - 4.9.1. Shot sequence showing immunofluorescence imaging being performed in the laboratory.
  - 4.9.2. Shot of RT-qPCR running.
  - 4.9.3. Shot of Western blot membrane imaging.

# Results

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## 5. Results

- 5.1. Following oxidative stress induction, tBHP treatment significantly reduced melanocyte proliferation [1] compared to controls [2].
  - 5.1.1. LAB MEDIA: Figure 2A. *Video editor: Emphasize the entire tBHP curve in the line graph.*
  - 5.1.2. LAB MEDIA: Figure 2A. *Video editor: Emphasize the entire Ctrl curve in the line graph.*
- 5.2. The treated cells reached only 1.4 cumulative population doublings or cPDL (C-P-D-L) by day 9 [1], whereas controls reached 3.1 cPDL [2]. By day 15, treated cells plateaued at 1.9 cPDL [3] while control cells expanded to 5.1 cPDL [4].
  - 5.2.1. LAB MEDIA: Figure 2A. *Video editor: Emphasize the tBHP line at the d9 point around 1.4 cPDL.*
  - 5.2.2. LAB MEDIA: Figure 2A. *Video editor: Emphasize the Ctrl line at the d9 point around 3.1 cPDL.*
  - 5.2.3. LAB MEDIA: Figure 2A. *Video editor: Emphasize the tBHP line at the d15 point around 1.9 cPDL.*
  - 5.2.4. LAB MEDIA: Figure 2A. *Video editor: Emphasize the Ctrl line at the d15 point around 5.1 cPDL.*
- 5.3. Melanocytes treated with tBHP showed increased cell surface area [1] compared to controls at all measured time points [2] with enlargement progressing until day 15 [3] consistent with the enlarged and flattened morphology of senescent cells [4].
  - 5.3.1. LAB MEDIA: Figure 2B. *Video editor: Emphasize all dark teal tBHP bars at day 4, day 9, and day 15.*
  - 5.3.2. LAB MEDIA: Figure 2B. *Video editor: Emphasize all light teal Ctrl bars at day 4, day 9, and day 15.*
  - 5.3.3. LAB MEDIA: Figure 2B. *Video editor: Emphasize the dark teal tBHP bar at day 15.*
  - 5.3.4. LAB MEDIA: Figure 2C. *Video editor: Emphasize the bottom image.*
- 5.4. tBHP-treated melanocytes showed an increase in senescence-associated beta-galactosidase or SA- $\beta$ -gal (S-A-beta-galactosidase) activity with 32% cells positive cells [1] compared 3% in controls [2]. The rise in SA- $\beta$ -gal positivity was consistent with a senescent phenotype [3].
  - 5.4.1. LAB MEDIA: Figure 2D. *Video editor: Emphasize the dark teal bar labelled as tBHP.*

- 5.4.2. LAB MEDIA: Figure 2D. *Video editor: Emphasize the light teal bar labelled as Ctrl.*
- 5.4.3. LAB MEDIA: Figure 2E: *Video editor: Emphasize the bottom image.*
- 5.5. Immunofluorescence analysis showed increased gamma-H2AX (*H-two-A-X*) levels in tBHP-treated melanocytes [1] compared with controls [2].
  - 5.5.1. LAB MEDIA: Figure 2G. *Video editor: Emphasize the dark teal bar labelled as tBHP.*
  - 5.5.2. LAB MEDIA: Figure 2G. *Video editor: Emphasize the light teal bar labelled as Ctrl.*
- 5.6. The treated cells showed stronger gamma-H2AX signal [1], than controls [2].
  - 5.6.1. LAB MEDIA: Figure 2F. *Video editor: In the tBHP row, emphasize the bright green spots under  $\gamma$ H2AX and merged.*
  - 5.6.2. LAB MEDIA: Figure 2F. *Video editor: In the Ctrl row, emphasize the bright green spots under  $\gamma$ H2AX and merged.*
- 5.7. Real Time-quantitative polymerase chain reaction analysis demonstrated increased senescence-associated secretory phenotype factor expression [1], with interleukin-8 showing the largest increase [2]. While interleukin-6 and matrix metalloproteinase-1 showed moderate increases [3].
  - 5.7.1. LAB MEDIA: Figure 2H. *Video editor: Emphasize all the dark teal coloured bars labelled as tBHP.*
  - 5.7.2. LAB MEDIA: Figure 2H. *Video editor: Emphasize the dark teal tBHP bar under IL-8.*
  - 5.7.3. LAB MEDIA: Figure 2H. *Video editor: Emphasize the dark teal tBHP bar under IL-6 and MMP-1.*
- 5.8. Western blot analysis confirmed senescence induction [1]. LaminB1 showed a significant reduction in tBHP-treated melanocytes [2] compared to controls [3].
  - 5.8.1. LAB MEDIA: Figure 2I.
  - 5.8.2. LAB MEDIA: Figure 2J. *Video editor: Emphasize the dark teal coloured bar labeled as tBHP.*
  - 5.8.3. LAB MEDIA: Figure 2J. *Video editor: Emphasize the light teal coloured bar labeled as Ctrl.*
- 5.9. Phosphorylated retinoblastoma protein levels were reduced in tBHP-treated melanocytes [1] compared to controls [2].
  - 5.9.1. LAB MEDIA: Figure 2K. *Video editor: Emphasize the dark teal coloured bar labeled as tBHP.*
  - 5.9.2. LAB MEDIA: Figure 2K. *Video editor: Emphasize the light teal coloured bar labeled as Ctrl.*

- 5.10. tBHP treatment increased transcriptional expression of p16 [1] and p21 in melanocytes [2].
  - 5.10.1. LAB MEDIA: Figure 2L. *Video editor: Emphasize the dark teal coloured bar labeled as tBHP under p16.*
  - 5.10.2. LAB MEDIA: Figure 2L. *Video editor: Emphasize the dark teal coloured bar labeled as tBHP under p21.*

1. Melanocytes  
Pronunciation link: <https://www.merriam-webster.com/dictionary/melanocyte>  
IPA: /məˈlænəˌsaɪt/  
Phonetic Spelling: muh·LAN·uh·site
2. Reagents  
Pronunciation link: <https://www.merriam-webster.com/dictionary/reagent>  
IPA: /riˈeɪdʒənt/  
Phonetic Spelling: ree·AY·juhnt
3. Confluency  
Pronunciation link: No confirmed link found  
IPA: /ˈkɑːnfluənsi/  
Phonetic Spelling: KAHN·floo·uhn·see
4. Aspirate  
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>  
IPA: /ˈæspəˌreɪt/  
Phonetic Spelling: AS·puh·rayt
5. Adherent  
Pronunciation link: <https://www.merriam-webster.com/dictionary/adherent>  
IPA: /ədˈhɪrənt/  
Phonetic Spelling: uhd·HEER·uhnt
6. Trypsin  
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>  
IPA: /ˈtrɪpsən/  
Phonetic Spelling: TRIP·suhn
7. EDTA  
Pronunciation link: <https://www.merriam-webster.com/dictionary/EDTA>  
IPA: /ˌiːˌdiːˌtiːˈeɪ/  
Phonetic Spelling: ee·dee·tee·AY
8. Incubator  
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>  
IPA: /ˈɪŋkjəˌbeɪtər/  
Phonetic Spelling: ING·kyuh·bay·ter
9. Celsius  
Pronunciation link: <https://www.merriam-webster.com/dictionary/Celsius>  
IPA: /ˈselsiəs/  
Phonetic Spelling: SEL·see·uhs

10. Detachment  
Pronunciation link: <https://www.merriam-webster.com/dictionary/detachment>  
IPA: /dɪˈtætʃmənt/  
Phonetic Spelling: dih·TACH·muhnt
11. Inhibitor  
Pronunciation link: <https://www.merriam-webster.com/dictionary/inhibitor>  
IPA: /ɪnˈhɪbətər/  
Phonetic Spelling: in·HIB·uh·ter
12. Suspension  
Pronunciation link: <https://www.merriam-webster.com/dictionary/suspension>  
IPA: /səˈspɛnʃən/  
Phonetic Spelling: suh·SPEN·shuhn
13. Conical  
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>  
IPA: /ˈkɑːnɪkəl/  
Phonetic Spelling: KAH·nih·kuhl
14. Centrifuge  
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>  
IPA: /ˈsentrəˌfjuːdʒ/  
Phonetic Spelling: SEN·truh·fyooj
15. Supernatant  
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>  
IPA: /ˌsuːpərˈneɪtənt/  
Phonetic Spelling: soo·per·NAY·tuhnt
16. Resuspend  
Pronunciation link: No confirmed link found  
IPA: /ˌriːsəˈspend/  
Phonetic Spelling: ree·suh·SPEND
17. Hydroperoxide  
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydroperoxide>  
IPA: /ˌhaɪdroʊpəˈrɑːksaɪd/  
Phonetic Spelling: hy·droh·puh·RAHK·side
18. Micromolar  
Pronunciation link: No confirmed link found  
IPA: /ˌmaɪkroʊˈmoʊlər/  
Phonetic Spelling: my·kroh·MOH·ler
19. Regimen  
Pronunciation link: <https://www.merriam-webster.com/dictionary/regimen>  
IPA: /ˈredʒəməɪn/  
Phonetic Spelling: REJ·uh·muhn
20. Senescence  
Pronunciation link: <https://www.merriam-webster.com/dictionary/senescence>  
IPA: /sɪˈnesəns/  
Phonetic Spelling: sih·NES·uhns

21. Formaldehyde  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/formaldehyde>  
 IPA: /fɔːrˈmældəˌhaɪd/  
 Phonetic Spelling: for·MAL·duh·hyde
22. Glutaraldehyde  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/glutaraldehyde>  
 IPA: /ˌɡluːtərˈældəˌhaɪd/  
 Phonetic Spelling: gloo·ter·AL·duh·hyde
23. Microscopy  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>  
 IPA: /maɪˈkraːskəpi/  
 Phonetic Spelling: my·KRAH·skuh·pee
24. Immunofluorescence  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/immunofluorescence>  
 IPA: /ˌɪmjənoʊfloʊˈresəns/  
 Phonetic Spelling: im·yuh·noh·floo·RES·uhns
25. RT-qPCR  
 Pronunciation link: No confirmed link found  
 IPA: /ˌɑːrˈtiːkjuːpiːsiːˈɑːr/  
 Phonetic Spelling: ar·tee·kyoo·pee·see·AR
26. Oxidative  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/oxidative>  
 IPA: /ˈɑːksəˌdeɪtɪv/  
 Phonetic Spelling: AHK·suh·day·tiv
27. Proliferation  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/proliferation>  
 IPA: /prəˌlɪfəˈreɪʃən/  
 Phonetic Spelling: pruh·lif·uh·RAY·shuhn
28. Morphology  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>  
 IPA: /mɔːrˈfɑːlədʒi/  
 Phonetic Spelling: mor·FAH·luh·jee
29. Senescent  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/senescent>  
 IPA: /sɪˈnesənt/  
 Phonetic Spelling: sih·NES·uhnt
30. Galactosidase  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/galactosidase>  
 IPA: /ɡəˌlæktˈɑːsəˌdeɪs/  
 Phonetic Spelling: guh·lak·TAH·suh·days
31. Phenotype  
 Pronunciation link: <https://www.merriam-webster.com/dictionary/phenotype>  
 IPA: /ˈfiːnəˌtaɪp/  
 Phonetic Spelling: FEE·nuh·type

32. Gamma-H2AX  
Pronunciation link: No confirmed link found  
IPA: /'gæmə ˌeɪtʃ ˌtuː ˌeɪ ˈeks/  
Phonetic Spelling: GAM·uh aych·too·ay·EKS
33. Polymerase  
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>  
IPA: /pəˈlɪməˌreɪs/  
Phonetic Spelling: puh·LIM·uh·rays
34. Interleukin  
Pronunciation link: <https://www.merriam-webster.com/dictionary/interleukin>  
IPA: /ˌɪntərˈluːkən/  
Phonetic Spelling: in·ter·LOO·kuhn
35. Metalloproteinase  
Pronunciation link: <https://www.merriam-webster.com/dictionary/metalloproteinase>  
IPA: /məˈtælouˈproʊtiˌneɪs/  
Phonetic Spelling: muh·tal·oh·PROH·tee·nays
36. LaminB1  
Pronunciation link: No confirmed link found  
IPA: /ˈlæmɪn ˈbiː ˈwʌn/  
Phonetic Spelling: LAM·in bee·wun
37. Phosphorylated  
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphorylate>  
IPA: /ˈfɑːsfəˌreɪtəd/  
Phonetic Spelling: FAH·sfuh·ray·tuhd
38. Retinoblastoma  
Pronunciation link: <https://www.merriam-webster.com/dictionary/retinoblastoma>  
IPA: /ˌretənəblæˈstoumə/  
Phonetic Spelling: ret·uh·nuh·bla·STOH·muh
39. Transcriptional  
Pronunciation link: <https://www.merriam-webster.com/dictionary/transcriptional>  
IPA: /trænˈskrɪpʃənəl/  
Phonetic Spelling: tran·SKRIP·shuh·nuhl