FINAL SCRIPT: APPROVED FOR FILMING

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Title: Measurement of Cyclic Guanosine Monophosphate (cGMP) in Solid Tissues Using Competitive Enzyme-Linked Immunosorbent Assay (ELISA)

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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? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 20
Number of Shots: 51

Introduction

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

1.1. **Haleigh Brown:** The Schechter laboratory researches nitric oxide metabolism. Nitric oxide stimulates cGMP formation through an adaptive homeostasis cascade. We aim to learn how aging, diet, and other factors affect this cascade **[1]**.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

What research gap are you addressing with your protocol?

1.2. **Haleigh Brown:** Previous research focuses on nitric oxide's formation and the resulting physiological effects while neglecting the cascade connecting the two. Our protocol measures cGMP, a second messenger in the cascade **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll: 4.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Haleigh Brown:** Our protocol is simple, accessible, and easily adaptable to various tissue types **[1]**.

1.3.1. 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 research has been approved by the Animal Care and Use Committee at National Institute of Diabetes and Digestive and Kidney Diseases

Protocol

2. Preparation, Homogenization, and Acetylation of Porcine Tissue Samples for ELISA Assay

Demonstrator: Haleigh Brown

2.1. To begin, place a tissue pulverizer, tweezers, and spoon on dry ice for 10 minutes **[1]**. Weigh out an excess of porcine tissue to account for loss during pulverization **[2]**. Transfer the tissue to the chilled pulverizer placed on dry ice **[3]**.

2.1.1. WIDE: Talent placing the tissue pulverizer, tweezers, and spoon onto dry ice.

2.1.2. Talent weighing a piece of tissue.

2.1.3. Talent transferring tissue to the pulverizer on dry ice.

2.2. Flash-freeze the tissue by pouring liquid nitrogen into the pulverizer and allow it to fully vaporize before proceeding **[1]**. Then replace the lid of the pulverizer and strike it 15 times using a mallet **[2]**.

2.2.1. Talent pouring liquid nitrogen onto the tissue in the pulverizer.

2.2.2. Talent hammering the lid of the pulverizer.

2.3. Now, tare a bead homogenizer tube on a balance **[1]**. Using the chilled spoon and tweezers, transfer the pulverized tissue into the tube **[2]**.

2.3.1. Talent taring a bead homogenizer tube on a balance.

2.3.2. Talent transferring the pulverized tissue into the tube using tweezers and a spoon.

2.4. Pipette 0.1 normal hydrochloric acid into the homogenization tube, ensuring the volume in microliters is a multiple of the tissue mass in milligrams **[1]**. Then, homogenize the tissue sample using a bead mill homogenizer **[2]**.

2.4.1. Talent pipetting hydrochloric acid into the tube labeled ‘P1 Ctrl Glut’.

2.4.2. Talent loading the tube into a bead mill homogenizer.

2.5. Centrifuge the homogenate at 17,000 *g* for 30 minutes while maintaining a sample temperature of 4 degrees Celsius **[1]**. Transfer the resulting supernatant to a

microcentrifuge tube **[2]**. Centrifuge again for 10 minutes while maintaining a sample temperature of 4 degrees Celsius **[3]**. Pipette an aliquot of the final supernatant into a microcentrifuge tube and place it on ice **[4]**.

2.5.1. Talent placing the tube into a centrifuge and setting the parameters.

2.5.2. Talent pipetting supernatant into a microcentrifuge tube.

2.5.3. Talent placing the tube into the centrifuge.

2.5.4. Talent pipetting an aliquot of supernatant into a microcentrifuge tube and placing it in a bucket of ice.

2.6. Next, pipette 9.9 milliliters of ELISA *(ih-LEE-zuh)* buffer into a tube labeled standard B **[1]**. Pipette 100 microliters of standard stock solution into the standard B tube **[2-TXT].** Vortex thoroughly to ensure an even suspension **[3]**.

2.6.1. Talent pipetting 9.9 milliliters of ELISA buffer into the tube labeled standard B.

2.6.2. Talent adding 100 microliters of standard stock to the standard B tube. **TXT: Standard stock: 300 pmol/mL of cGMP**

2.6.3. Talent vortexing the tube contents.

2.7. Transfer 1,000 microliters of the solution from standard B to the tube labeled standard 1 **[1]**. Then add 500 microliters of ELISA buffer to the tubes labeled standard 0 and standards 2 through 8 **[2]**.

2.7.1. Talent pipetting 1,000 microliters from standard B into standard 1.

2.7.2. Talent adding 500 microliters of ELISA buffer into standard 0 and all standards 2 through 8.

2.8. Vortex standard 1 **[1]**, then transfer 500 microliters of solution into standard 2 **[2]**. Then vortex standard 2 **[3]** and transfer 500 microliters of the solution into standard 3 **[4]**.

2.8.1. Talent vortexing standard 1 .

2.8.2. Talent transferring 500 microliters of solution in standard 1 into standard 2.

2.8.3. Talent vortexing standard 2.

2.8.4. Talent transferring 500 microliters from standard 2 into standard 3.

2.9. Continue transferring 500 microliters from each previous standard into the next, vortexing thoroughly after each addition, until standard 8 is prepared **[1]**.

2.9.1. Talent completing serial dilutions from standard 3 through standard 8.

2.10. For the acetylation of the samples, add 50 microliters of 4 molar potassium hydroxide to a 250-microliter sample aliquot **[1]**. Immediately add 12.5 microliters of acetic anhydride **[2]**.

2.10.1. Talent pipetting potassium hydroxide into a tube with 250 microliter sample aliquot.

2.10.2. Shot of 12.5 µL acetic anhydride being added to the tube.

2.11. Vortex the solution for 15 seconds **[1]**. Then pipette 12.5 microliters of 4 molar potassium hydroxide and vortex again **[2]**.

2.11.1. Talent vortexing the sample tube for 15 seconds.

2.11.2. Talent adding potassium hydroxide to the sample and vortexing.

2.12. To the tube labeled standard 0, add 100 microliters of 4 molar potassium hydroxide **[1]** followed immediately by 25 microliters of acetic anhydride **[2-TXT]**. Then pipette 25 microliters of 4 molar potassium hydroxide to the tube and vortex briefly **[3]**.

2.12.1. Talent pipetting 100 microliters of potassium hydroxide into standard 0.

2.12.2. Talent pipetting 25 microliters of acetic anhydride into standard 0. **TXT: Vortex for 15 s**

2.12.3. Talent pipetting potassium hydroxide and vortexing standard 0.

2.13. Repeat the potassium hydroxide and acetic anhydride additions, followed by vortexing, for the remaining standards **[1]**.

2.13.1. Shot of all tubes after the addition of KOH and acetic anhydride.

3. ELISA Plate Setup, Incubation, and Detection Using Acetylcholinesterase (AChE) Tracer and Ellman's Reagent

3.1. Add a pre-determined ratio of ELISA buffer and acetylated sample aliquot to a microcentrifuge tube **[1-TXT]**. Vortex standard 0 **[2]**. Transfer 50 microliter aliquots of standard 0 to five wells designated as the NSB *(N-S-B)* and B_0 *(B-nought)* wells of the ELISA microplate **[3]**. Then add 50 microliters of ELISA buffer into the 2 NSB wells containing standard 0 **[4]**.

3.1.1. Talent pipetting ELISA buffer and sample into a microcentrifuge tube. **TXT: Ratio (Sample: ELISA Buffer): 1:2 or 1:3**

Video Editor: The text overlay has a ratio

3.1.2. Talent vortexing standard 0 before use.

3.1.3. Talent pipetting 50 microliters of standard 0 into five wells on the ELISA plate.

3.1.4. Talent pipetting ELISA buffer into the two NSB wells.

3.2. Using a single pipette tip, add 50 microliter aliquots of standards 1 through 8 in duplicate wells, beginning with standard 8 and proceeding to standard 1 **[1]**. Vortex each sample **[2]**, then add 50 microliters into the appropriate wells in duplicate or triplicate **[3]**.

3.2.1. Talent pipetting standards into ELISA plate in duplicate, starting with most dilute.

3.2.2. Talent vortexing samples.

3.2.3. Talent pipetting vortexed samples into the wells accordingly.

3.3. Now pipette 50 microliters of acetylcholinesterase tracer to all wells designated as NSB, B_0, standard, and sample **[1]**. Add 50 microliters of antiserum to each B_0, standard, and sample well **[2]**.

3.3.1. Talent pipetting AChE tracer into all relevant wells.

3.3.2. Talent pipetting antiserum into appropriate wells.

3.4. Cover the ELISA plate and incubate at 4 degrees Celsius for 18 hours **[1]**. After incubation, invert the ELISA plate over a paper towel to dispose of the contents **[2].** Fill the wells with wash buffer using a wash bottle **[3]**.

3.4.1. Talent covering the plate and placing it into a refrigerator or cold chamber.

3.4.2. Talent inverting and tapping the ELISA plate to empty the wells.

3.4.3. Talent filling ELISA wells with wash buffer.

3.5. Gently agitate for 5 seconds **[1]**, then dump the wash buffer and tap out the remaining liquid on a paper towel **[2-TXT]**.

3.5.1. Talent agitating the ELISA plate.

3.5.2. Shot of the plate being inverted and tapped over a paper towel. **TXT: Repeat wash cycle 4 times with 30 s of agitation**

3.6. Now, transfer Ellman's reagent to a reservoir **[1]**. Working quickly, use a multichannel pipette to add 200 microliters of Ellman's reagent to the blank, NSB, B0, TA, standard, and sample wells **[2]**.

3.6.1. Talent pouring Ellman's reagent into a reagent reservoir.

3.6.2. Talent rapidly pipetting Ellman's reagent across all relevant wells.

3.7. Add 5 microliters of acetylcholinesterase tracer to the TA *(T-A)* well **[1]**. Then cover the ELISA plate with parafilm **[2].** Place it in a light-protected container to incubate at room temperature with agitation for 60 to 90 minutes **[3]**. Remove the parafilm and read the optical density at 412 nanometers **[4]**.

3.7.1. Talent pipetting AChE tracer into the TA well.

3.7.2. Shot of the plate being sealed with parafilm.

3.7.3. Talent placing sealed plate in a light protected container, in a incubator with agitation.

3.7.4. Talent removing parafilm and placing plate into a plate reader set to 412 nanometers.

Results

4. Results

4.1. cGMP *(Cyclic-G-M-P)* concentration in porcine liver at baseline was approximately 0.0202 nanomoles per gram of tissue **[1].**

4.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bar labeled "Baseline"*

4.2. In the nitrate-treated group, cGMP concentration increased to approximately 0.0364 nanomoles per gram of tissue **[1].**

4.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bar labeled "Nitrate"*

Pronunciation Guide:

1. pulverizer

No confirmed link found
IPA: /ˈpʌlvəˌraɪzər/
Phonetic Spelling: PUL-vuh-rie-zur

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2. porcine

Pronunciation link: https://www.merriam-webster.com/dictionary/porcine youtube.com+8justpronounce.com+8howtopronounce.com+8merriam-webster.com+1merriam-webster.com+1merriam-webster.com+12merriam-webster.com+12collinsdictionary.com+12
IPA: /ˈpɔːr.saɪn/
Phonetic Spelling: por-syne

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3. homogenizer

No confirmed link found
IPA: /həˈmɒdʒəˌnaɪzər/
Phonetic Spelling: huh-MODH-uh-nie-zur

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4. hydrochloric

No confirmed link found
IPA: /ˌhaɪdrəˈklɔrɪk/
Phonetic Spelling: hy-DRUH-klor-ik

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5. microliters

No confirmed link found
IPA: /ˈmaɪkrəˌliːtərz/
Phonetic Spelling: MY-kruh-lee-turz

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6. centrifuge

No confirmed link found
IPA: /ˈsɛntrɪˌfjuːdʒ/
Phonetic Spelling: SEN-tri-fyooj

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7. supernatant

No confirmed link found
IPA: /ˌsuːpərˈneɪtənt/
Phonetic Spelling: SOO-pur-NAY-tunt

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8. aliquot

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IPA: /ˈælɪkwɒt/
Phonetic Spelling: AL-i-kwot

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9. ELISA

No confirmed link found
IPA: /ɪˈliːzə/
Phonetic Spelling: ih-LEE-zuh

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10. acetylation

No confirmed link found
IPA: /əˌsɛtəˈleɪʃən/
Phonetic Spelling: uh-SET-uh-LAY-shun

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11. potassium hydroxide

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IPA: /pəˈtæsiəm ˌhaɪdrɒkˈsaɪd/
Phonetic Spelling: puh-TAS-ee-um hy-drok-SIDE

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12. acetic anhydride

Pronunciation link: https://www.merriam-webster.com/dictionary/acetic%20anhydride dictionary.cambridge.org+1dictionary.cambridge.org+1howtopronounce.commerriam-webster.com+12merriam-webster.com+12synonyms.com+12howtopronounce.com+15merriam-webster.com+15dictionary.cambridge.org+15
IPA: /əˈsiːtɪk ænˈhaɪdraɪd/
Phonetic Spelling: uh-SEET-ik an-HY-dryde

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13. acetylcholinesterase

Pronunciation link: https://www.merriam-webster.com/dictionary/acetylcholinesterase merriam-webster.com+12merriam-webster.com+12definitions.net+12
IPA: /əˌsɛtəlˌkoʊlɪˈnɛstərˌeɪs/
Phonetic Spelling: uh-SET-uhl-COH-lih-NES-tur-ays

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14. Ellman's (as in Ellman's reagent)

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IPA: /ˈɛlmənz/
Phonetic Spelling: EL-mans

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15. nanometers

No confirmed link found
IPA: /ˈnænəˌmiːtərz/
Phonetic Spelling: NAN-uh-mee-terz
