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# Title: Tear-Derived Exosomal miR-15a as New Diagnostic Tool for Diabetic Retinopathy

## 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? **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? **Yes**

   How far apart are the locations? **500m**

**Current Protocol Length**
Number of Steps: 30
Number of Shots: 47

# Introduction

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

1.1. **Musfirah Mahmud:** Our research explores the potential of tear-derived exosomal miR-15a as a non-invasive biomarker for diabetic retinopathy, aiming to improve early diagnosis and patient outcomes in diabetes-related eye disease.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.10.1.*

What technologies are currently used to advance research in your field?

1.2. **Musfirah Mahmud:** Current research leverages droplet digital PCR, exosome isolation, and molecular profiling of tear-based microRNAs to enhance diagnostic accuracy and biomarker discovery for diabetic retinopathy.

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

What are the current experimental challenges?

1.3. **Tengku Ain Kamalden:** Current challenges include limited tear volume, absence of specialized tear isolation kits, and lack of a validated endogenous reference miRNA, complicating normalization and consistent quantification in tear-based diagnostics.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.1.*

What significant findings have you established in your field?

1.4. **Sujaya Singh:** We identified tear-derived extracellular vesicular or exosomal biomarkers as a non-invasive tool for diabetic retinopathy. Though DR and non-DR groups overlapped, findings support tear fluid's potential for early disease detection.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 4.*

What research questions will your laboratory focus on in the future?

1.5. **Tengku Ain Kamalden:** We will focus on how other miRNAs regulate diabetic retinopathy, explore their use as a non-invasive biomarker, and investigate their role in other eye diseases to prevent vision loss.

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:**

***Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.***

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Tengku Ain Kamalden, Professor, Universiti Malaya**: Publishing with JoVE will enhance our research visibility by providing clear, visual demonstrations of our methods, making complex techniques accessible to a wider audience. This increases reproducibility, fosters collaboration, and accelerates adoption of our findings, ultimately boosting the impact and reach of our work within the scientific community.

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

**Ethics Title Card**

This research has been approved by the Universiti Malaya Medical Centre Medical Research Ethical Committee

# Protocol

## 2. Pre-Processing of Tear Samples and Exosome Isolation

**Demonstrator:** Musfirah Mahmud

2.1. To begin, take a tube containing the Schirmer strip soaked in PBS **[1]** and vortex it for 5 minutes at room temperature **[2]**.

2.1.1. WIDE: A shot of the tube with the Schirmer strip soaked in PBS and the talent holding the tube or standing beside it.

2.1.2. Talent vortexing the tube.

2.2. Place the tube on a rocker and agitate it for 5 minutes at room temperature **[1]**.

2.2.1. Talent placing the tube onto a rocking platform and starting the agitation.

2.3. Now, centrifuge the tube at 2000 *g* for 15 minutes at 4 degrees Celsius **[1]**.

2.3.1. Talent placing the tube into a centrifuge and closing the lid to begin centrifugation.

2.4. Transfer the solution into a new labeled tube **[1]** and store it at minus 80 degrees Celsius until further processing **[2]**.

2.4.1. Talent using a pipette to transfer the supernatant into a clean, labeled tube.

2.4.2. Talent placing the labeled tube into a minus 80 degrees Celsius freezer.

2.5. For the exosome isolation, thaw the tear solution on ice for 10 minutes **[1]**.

2.5.1. Talent placing the tear solution on ice for thawing.

2.6. Equilibrate the spin column for 15 minutes at room temperature **[1]**.

2.6.1. Talent placing the spin column on the bench.

2.7. Then, remove the lower outlet of the column **[1]** and position it over the provided waste collection plate **[2]**.

2.7.1. Talent detaching the lower outlet of the column.

2.7.2. Talent mounting the column onto the waste collection plate.

2.8. Remove the top sealing mat and allow the storage buffer to pass through the column by gravity **[1]**.

2.8.1. Talent removing the top sealing mat and the storage buffer passing through the column by gravity.

2.9. Then, add 250 microliters of PBS and let it pass through the column **[1]**.

2.9.1. Talent pipetting PBS onto the column and PBS passing through the column.

2.10. Apply 110 microliters of a sample onto the top of each column and allow it to enter the column **[1]**.

2.10.1. Talent pipetting the tear sample onto the top of the column and the sample entering the column.

2.11. Move the column onto a provided sample collection plate **[1]**, add 100 microliters of PBS to the top, and allow it to pass through the column **[2]** into the sample collection plate **[3].**

2.11.1. Talent transferring the spin column onto a sample collection plate.

2.11.2. Talent adding PBS to the column and PBS passing through it.

2.11.3. A shot of PBS collected into the sample collection plate.

2.12. Place the column back onto the waste collection plate **[1]**.

2.12.1. Talent reattaching the column to the waste collection plate.

2.13. Add 200 microliters of PBS four times to the column and allow it to enter the column to remove the free protein fraction from the first loading **[1]**.

2.13.1. Talent adding PBS to the column and PBS flowing through the column.

2.14. Then, add 110 microliters of the sample into the column and allow it to enter **[1]**.

2.14.1. Talent adding sample to the column and the sample entering the column.

2.15. Place the column onto the sample collection plate **[1]**, add 100 microliters of PBS, and allow it to pass through the column **[2]**.

2.15.1. Talent transferring column onto collection plate.

2.15.2. Talent pipetting PBS to the column and PBS passing through the column.

2.16. Briefly centrifuge the sample collection plate at 100 *g* for 30 seconds **[1]**. The isolated exosomes are now ready for RNA isolation **[2]**.

2.16.1. Talent placing the sample collection plate into a centrifuge and initiating the spin.

2.16.2. A shot of the isolated exosomes.

## 3. RNA Isolation

3.1. Take 200 microliters of the isolated exosomes **[1]** and add 60 microliters of lysis buffer to it **[2]**. Vortex for 5 seconds **[3]** and incubate the mixture at room temperature for 3 minutes **[4]**.

3.1.1. Talent pipetting 200 microliters of the isolated exosomes into a tube.

3.1.2. Talent pipetting lysis buffer into the exosome.

3.1.3. Talent placing the tube on a vortex mixer.

3.1.4. Talent placing the tube on the bench.

3.2. Add 20 microliters of inhibitor precipitation buffer **[1]**, vortex again for 20 seconds before incubating for 3 minutes at room temperature **[2]**.

3.2.1. Talent pipetting the inhibitor precipitation buffer into the tube containing the exosomes-lysis buffer mixture.

3.2.2. Talent vortexing the tube.

3.3. Centrifuge the tube at 12,000 *g* for 3 minutes at room temperature **[1]**.

3.3.1. Talent loading the tube into the centrifuge.

3.4. Transfer the clear, colorless supernatant to a new tube **[1]** and add one volume of isopropanol to it **[2]**.

3.4.1. Talent pipetting the clear supernatant into a fresh, labeled tube.

3.4.2. Talent adding an equal volume of isopropanol to the tube containing the supernatant.

3.5. After vortexing for 5 seconds, transfer the solution to the mini spin column provided in the kit **[1]**.

3.5.1. Talent pipetting the mixture into a mini spin column.

3.6. Centrifuge the column at room temperature for 15 seconds at 8000 *g* **[1]** and discard the flowthrough **[2]**.

3.6.1. Talent placing the mini column in a centrifuge.

3.6.2. Talent disposing of the flowthrough.

3.7. Wash the column with 700 microliters of RWT *(R-W-T)* wash buffer **[1-TXT]**.

3.7.1. Talent pipetting RWT buffer into the column. **TXT: Centrifuge for 15 s, 8000 x *g*, RT**

3.8. After centrifuging for 15 seconds, discard the flowthrough **[1-TXT]**.

3.8.1. Talent discarding the flowthrough (obtained after centrifugation).

3.9. Now, wash the column with 500 microliters of RPE wash buffer before centrifuging again for 15 seconds **[1]**.

3.9.1. Talent adding RPE buffer to the column.

3.10. Discard the flowthrough **[1]** and add 500 microliters of 80 percent ethanol to the sample mini spin column before centrifuging again **[2-TXT]**

3.10.1. Talent discarding the flowthrough.

3.10.2. Talent pipetting ethanol into the sample mini spin column. **TXT: Centrifugation: 2 min, 8000 x *g*, RT**

3.11. After discarding the flowthrough and the collection tube, place the mini spin column into a new 2 milliliter collection tube **[1]**.

3.11.1. Talent inserting the column into a fresh 2-milliliter collection tube.

3.12. Centrifuge at 18,000 *g* for 5 minutes **[1]** and discard the flowthrough and collection tube **[2]**.

3.12.1. Talent placing the setup in a centrifuge.

3.12.2. Talent discarding the flowthrough and collection tube.

3.13. Place the mini spin column into a new 1.5-milliliter tube **[1]**, then add 15 microliters of RNase-free water at the center of the spin column membrane **[2]**.

3.13.1. Talent positioning the spin column in a 1.5-milliliter microcentrifuge tube.

3.13.2. Talent applying RNase-free water to the center of the membrane.

3.14. After incubating for 3 minutes at room temperature, centrifuge the tube at 18,000 *g* for 1 minute **[1]** to elute the RNA **[2]**.

3.14.1. Talent centrifuging the tube.

3.14.2. A shot of the collected eluate.

# Results

## 4. Results

4.1. Nanoparticle tracking analysis was used to evaluate the size distribution and concentration of exosomes isolated from tear samples **[1]**.

4.1.1. LAB MEDIA: Figure 3. *Video Editor: Only show the two graphs.*

4.2. The average size of exosomes isolated from tears was approximately 142.4 nanometers **[1]**.

4.2.1. LAB MEDIA: Figure 3. *Video Editor: Show the entire Figure 3. Highlight the line "Mean: 142.4 nm" in the top left table.*

4.3. The total number of exosomes in the sample diluted to 1:20 *(one to twenty)* was 3.9 x $10^{10}$ *(three point nine times ten to the tenth)* particles per milliliter **[1-TXT]**.

4.3.1. LAB MEDIA: Figure 3. **TXT: Dilution: 1:20** *Video Editor: The onscreen text is a ratio. Show the entire Figure 3. Highlight the line in the bottom left table showing "Concentration: 1.95e+09 ± 1.55e+08 particles/mL".*

4.4. The copy number of microRNA-15a *(Micro-R-N-A fifteen-A)* significantly increased in patients with diabetes without retinopathy compared to healthy controls, suggesting early elevation of this biomarker with diabetic onset **[1]**.

4.4.1. LAB MEDIA: Figure 4. *Video Editor: Highlight the bar labeled "DM No DR".*

4.5. A further significant increase in microRNA-15a copy number was observed in patients with diabetic retinopathy compared to controls, reinforcing its association with diabetic conditions **[1]**.

4.5.1. LAB MEDIA: Figure 4. *Video Editor: Highlight the bar labeled "DR".*

4.6. No statistically significant difference in microRNA-15a levels was found between the diabetes without retinopathy and diabetic retinopathy groups, indicating that while miR-15a reflects diabetic presence, it does not differentiate between stages of retinopathy **[1]**.

4.6.1. LAB MEDIA: Figure 4. *Video Editor: Highlight the bars labeled "DM No DR" and "DR".*

**Pronunciation Guide:**

**1. Exosome**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/exosome
- **IPA**: /ˈɛk.səˌsoʊm/
- **Phonetic Spelling**: ek-suh-sohmmerriam-webster.com

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**2. microRNA**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/microRNA
- **IPA**: /ˌmaɪ.kroʊˌɑrˌɛnˈeɪ/
- **Phonetic Spelling**: my-kroh-ar-en-ay

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**3. Isopropyl Alcohol**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/isopropyl%20alcohol
- **IPA**: /ˌaɪ.səˈproʊ.pəl ˈæl.kəˌhɔl/
- **Phonetic Spelling**: eye-suh-proh-puhl al-kuh-hawlmerriam-webster.com+5merriam-webster.com+5merriam-webster.com+5

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**4. Lysis**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/lysis
- **IPA**: /ˈlaɪ.sɪs/
- **Phonetic Spelling**: ly-sismerriam-webster.com+9merriam-webster.com+9merriam-webster.com+9

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**5. Polymerase Chain Reaction (PCR)**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/polymerase%20chain%20reaction
- **IPA**: /pəˈlɪ.məˌreɪs tʃeɪn riˈæk.ʃən/
- **Phonetic Spelling**: puh-lim-uh-race chain ree-ak-shunmerriam-webster.com+1merriam-webster.com+1

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**6. Extracellular**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/extracellular
- **IPA**: /ˌɛk.strəˈsɛl.jə.lɚ/
- **Phonetic Spelling**: ek-struh-sell-yuh-lurmerriam-webster.com

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**7. Ribonucleotide**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/ribonucleotide
- **IPA**: /ˌraɪ.boʊˈnu.kli.əˌtaɪd/
- **Phonetic Spelling**: ry-boh-noo-klee-uh-tydmerriam-webster.com

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**8. Schirmer Strip**

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˈʃɪr.mɚ strɪp/
- **Phonetic Spelling**: shur-mer stripmerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3merriam-webster.com+1merriam-webster.com+1

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**9. RNase-Free Water**

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌɑrˌɛnˈeɪz fri ˈwɔ.tɚ/
- **Phonetic Spelling**: ar-en-aze free wah-ter

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**10. Nanoparticle**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/nanoparticle
- **IPA**: /ˈnæn.oʊˌpɑr.tɪ.kəl/
- **Phonetic Spelling**: nan-oh-par-ti-kl

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**11. Centrifuge**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/centrifuge
- **IPA**: /ˈsɛn.trəˌfjuːdʒ/
- **Phonetic Spelling**: sen-truh-fyooj

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**12. Vortex**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/vortex
- **IPA**: /ˈvɔr.tɛks/
- **Phonetic Spelling**: vor-teks

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**13. Pipette**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/pipette
- **IPA**: /ˌpaɪˈpɛt/
- **Phonetic Spelling**: pie-pet

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**14. Agitate**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/agitate
- **IPA**: /ˈædʒ.ɪ.teɪt/
- **Phonetic Spelling**: aj-ih-tayt

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**15. Supernatant**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/supernatant
- **IPA**: /ˌsuː.pɚˈneɪ.tənt/
- **Phonetic Spelling**: soo-per-nay-tuhnt

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**16. Incubate**

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

- **IPA**: /ˈɪn.kjəˌbeɪt/
- **Phonetic Spelling**: in-kyuh-bayt

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**17. Eluate**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/eluate
- **IPA**: /ˈɛl.ju.eɪt/
- **Phonetic Spelling**: el-yoo-atemerriam-webster.com+2merriam-webster.com+2merriam-webster.com+2merriam-webster.com

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**18. Nanometer**

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/nanometer
- **IPA**: /ˈnæn.oʊˌmiː.tɚ/
- **Phonetic Spelling**: nan-oh-mee-ter

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**19. MicroRNA-15a**

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌmaɪ.kroʊˌɑrˌɛnˈeɪ fɪfˈtiːn eɪ/
- **Phonetic Spelling**: my-kroh-ar-en-ay fifteen ay

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**20. miR-15a**

- **Pronunciation link**: No confirmed link found
- **IPA**: /ˌmaɪ.ɑr fɪfˈtiːn eɪ/
- **Phonetic Spelling**: my-ar fifteen ay