Submission ID #: 61559
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18775543

Title: Isolation of Mouse Kidney-Resident CD8+ T cells for Flow Cytometry Analysis

Authors and Affiliations:

Wei Liao[1,2], Chaoyu Ma[1], Nu Zhang[1]

[1]Department of Microbiology, Immunology and Molecular Genetics, School of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, 78229
[2]Department of Dermatology, Hunan Key Laboratory of Medical Epigenomics, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

Corresponding Authors:

Nu Zhang (zhangn3@uthscsa.edu)

Email Addresses for All Authors:

liaow@uthscsa.edu
mac4@uthscsa.edu
zhangn3@uthscsa.edu

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **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: 13
Number of Shots: 33

Videographer NOTE: Shot out of order

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Nu Zhang:** $CD8^+$ T cells carry distinct features in different tissues. As a critical example of non-mucosal and non-lymphoid tissues, it is essential to directly characterize kidney-resident cells to fully understand T cell biology.

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

1.2. **Nu Zhang:** Here, we will demonstrate a convenient method to isolated kidney-resident $CD8^+$ T cells accommodating subsequent flow cytometry analysis.

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

OPTIONAL:

1.3. **Nu Zhang:** This method could provide insight into the studies of effector and memory $CD8^+$ T cells generated after infections as well as autoimmune responses.

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

Introduction of Demonstrator on Camera:

1.4. **Nu Zhang:** The procedure will be performed by Dr. Chaoyu Ma, a research scientist from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at UT Health San Antonio.

Protocol

2. Intravascular Labeling of $CD8^+$ T Cells

2.1. Begin by diluting biotin anti-CD8-alpha antibody to 15 micrograms per milliliter in PBS, making sure that there is enough to administer 200 microliters to each mouse **[1]**. Prior to injection, heat the tail vein of the mouse with an overhead heat lamp for 5 to 10 minutes to dilate it **[2]**.

2.1.1. WIDE: Establishing shot of talent diluting the antibody.

2.1.2. Talent heating the tail vein under a lamp.

2.2. Draw 200 microliters of the prediluted antibody mix into a 28-gauge insulin syringe and remove any air bubbles by moving the piston up and down **[1]**. Bend the needle to create a 150-degree angle between the needle and the syringe, bevel up, so that the needle is parallel to the tail vein **[2]**.

2.2.1. Talent drawing up the antibody mix and then moving the piston up and down.

2.2.2. Talent bending the needle.

2.3. Restrain the mouse with a rodent restrainer **[1]** and spray the tail with 70% ethanol to make the vein clearly visible **[2]**. Hold the tail at the distal end with the thumb and middle fingers of the non-dominant hand, then place the index finger underneath the injection site **[3]**.

2.3.1. Talent putting the mouse in the restrainer.

2.3.2. Talent spraying the tail with ethanol. NOTE: This and next shot merged

2.3.3. Talent positioning the tail for injection.

2.4. With the dominant hand, insert the needle into the vein in parallel towards the direction of the heart and slowly inject 200 microliters of the antibody **[1]**. Remove the needle and gently compress the injection site until bleeding stops **[2]**.
Videographer: This step is important!

2.4.1. Talent inserting the needle into the tail and injecting the antibody. NOTE: This and next shot merged

2.4.2. Talent removing the needle and compressing the injection site.

2.5. After euthanizing the mouse, dissect the kidney with scissors **[1]** and transfer it to a 1.5-milliliter microcentrifuge tube **[2]**. Leave the samples on ice until further processing **[3]**.

2.5.1. Talent dissecting the kidney.

2.5.2. Talent putting the kidney in a microcentrifuge tube. NOTE: This and next shot merged

2.5.3. Talent putting the sample tube on ice.

3. Enzymatic Digestion of the Kidney

3.1. Prepare 6-well plates with 3 milliliters of digestion solution in each well, using 1 well per mouse, and store them on ice **[1]**. Add 300 microliters of digestion solution into each sample tube **[2]** and mince the kidney samples with a straight spring scissor **[3]**. *Videographer: This step is important!*

3.1.1. Talent placing a prepared plate on ice.

3.1.2. Talent adding digestion solution into a sample tube.

3.1.3. Talent mincing a kidney sample.

3.2. Transfer the minced kidney samples to the 6-well plates with the digestion solution **[1]**. Incubate the samples at 37 degrees Celsius with gentle rocking for 45 minutes **[2]**, then homogenize the tissue with the plunger flange of a 3-milliliter syringe **[3]** and transfer it to 15-milliliter conical tubes **[4]**.

3.2.1. Talent transferring the minced kidneys to the plate.

3.2.2. Plate rocking in the incubator.

3.2.3. Talent homogenizing the tissue.

3.2.4. Talent transferring a sample to the conical tube.

3.3. Spin the samples down at 500 *x g* and 4 degrees Celsius for 5 minutes **[1]**. Remove the supernatant and resuspend the pellet in 3 milliliters of RPMI with 10% FCS **[2]**.

3.3.1. Talent putting the conical tube in the centrifuge and closing the lid.

3.3.2. Talent resuspending the pellet in RPMI, with the RPMI container in the shot.

4. Density Gradient Centrifugation to Enrich Lymphocytes from the Digested Kidney

4.1. Spin down the sample at 500 *x g* and 4 degrees Celsius for 5 minutes **[1]**, then remove the supernatant **[2]** and resuspend the cell pellet with 5 milliliters of 44% density gradient medium and RPMI mix **[3]**. *Videographer: This step is important!*

4.1.1. Talent putting the sample tube in the centrifuge and closing the lid.

4.1.2. Talent removing the supernatant.

4.1.3. Talent resuspending the cell pellet, with the gradient medium container in the shot.

4.2. Put the tip of a 3-milliliter pipette containing 3 milliliters of 67% density gradient medium and PBS directly to the bottom of each tube and slowly release the solution

so that the heavy solution forms a distinct layer at the bottom **[1]**. *Videographer: This step is important!*

4.2.1. Talent adding the heavy solution to the bottom of the tube.

4.3. Spin the samples at 900 *x g* for 20 minutes with reduced accelerator and brake setting **[1]**, then carefully remove the tubes from the centrifuge without disturbing the layers **[2]**.

4.3.1. Talent putting the tube in the centrifuge and closing the lid.

4.3.2. Talent carefully taking the tube out of the centrifuge, with the layers visible.

4.4. Remove the top layer with a transfer pipette without touching the lymphocyte layer **[1]** and transfer the lymphocyte layer to a new 15-milliliter tube **[2]**. Fill the tube with PBS and 5% FCS **[3]**, then mix by slowly inverting the tube 4 to 6 times **[4]**. *Videographer: This step is important!*

4.4.1. Talent removing the top layer.

4.4.2. Talent transferring the lymphocyte layer to a new tube.

4.4.3. Talent filling the tube with PBS/5% FCS.

4.4.4. Talent inverting the tube.

4.5. After centrifuging the samples at 500 *x g* and 4 degrees Celsius for 5 minutes, remove the supernatant **[1]** and re-suspend the cell pellet with 500 microliters of complete RPMI medium. The cells are now ready for flow cytometry staining **[2]**.

4.5.1. Talent removing the supernatant.

4.5.2. Talent resuspending the cell pellet.

Results

5. Results: Flow Cytometry Analysis of Kidney Lymphocytes

5.1. Even after density centrifugation-mediated lymphocyte enrichment, it was very common to see a large portion of non-lymphocytes in the final product **[1]**. However, after gating on live lymphocytes, CD8-positive T lymphocytes were easy to identify **[2]**.

5.1.1. LAB MEDIA: Figure 1 B.

5.1.2. LAB MEDIA: Figure 1 B. *Video Editor: Emphasize the last two plots in the top row, live cells and CD8^{+}.*

5.2. As expected, only extravascular CD8-positive T cells efficiently acquired tissue-resident memory T cell phenotypes, such as upregulation of CD69 and downregulation of Ly6C **[1]**.

5.2.1. LAB MEDIA: Figure 1 B. *Video Editor: Emphasize the Ly6C and CD69 plots.*

5.3. In contrast to the infected mice, the vast majority of CD8-positive T cells isolated from young naïve mice housed in the SPF facility were labeled with CD8-alpha antibody and therefore belonged to the intravascular compartment **[1]**.

5.3.1. LAB MEDIA: Figure 1 C.

Conclusion

6. Conclusion Interview Statements

6.1. **Nu Zhang:** This technique has greatly accelerated the investigation of tissue-resident immune cells in densely vascularized organs.

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