

Single Nuclei Isolation from Embryonic Chicken Eye



Developed by Jared Tangeman from the Del Rio-Tsonis Lab at Miami University

About [the Del Rio-Tsonis Lab](#)

The Del Rio-Tsonis Lab explores eye regeneration, focusing on pigment epithelial cells reprogramming into lens and retina cells. Using models such as chicks and salamanders, the team investigates key genes, signals, and epigenetic regulators driving this process. Their ultimate goal is to apply these insights to retinal degenerative diseases.

This protocol was used in

Tangeman, J.A. et al. Integrated single-cell multiomics uncovers foundational regulatory mechanisms of lens development and pathology. *Development* 1 January 2024; 151 (1): dev202249. doi: <https://doi.org/10.1242/dev.202249>

Notice

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Materials

Materials	Supplier	Part Number	Notes
Hoechst-33342	Thermo Fisher Scientific [®]	H3570	
20 µm strainer	pluriSelect [®]	43-10020-40	
Tris-HCl	Millipore Sigma [®]	T2194	or equivalent
NaCl	Millipore Sigma	59222C	or equivalent
MgCl ₂	Millipore Sigma	M1028	or equivalent
Surfact-Amps-NP-40	Thermo Fisher Scientific	28324	
Nuclease-free water	Varies	Varies	
1X PBS	Gibco [®]	10-010-031	Or equivalent
10% BSA (Fraction V)	Varies	Varies	
RNase Inhibitor	Promega [®]	12615	
Centrifuge with swinging bucket rotor	Varies	Varies	Compatible with 15 mL or 5 mL centrifuge tubes and capable of reaching 4°C.
Fire-polished silanized pasteur pipette or Regular-bore pipette tips	Varies	Varies	

Hemocytometer	Varies	Varies	
Epifluorescence microscope	Varies	Varies	20x and 40x objectives
DAPI or AOPI	Varies	Varies	Optional nuclear stains

Preparation

1. Prepare the following solutions and add the reagents in the order provided in the table. Keep them on ice.
2. Clean the bench top and dissection area with 70% ethanol, followed by RNaseZAP to remove RNases.

Cell Lysis Buffer

Reagents	Stock Concentration	Final Concentration	Total Volumes for 10 mL
Tris-HCL	1 M	10 mM	100 μ L
NaCl	1 M	10 mM	100 μ L
MgCl ₂	1 M	3 mM	30 μ L
Surfact-Amps-NP-40	10%	0.01%	10 μ L
RNase Inhibitor	40 U/ μ L	0.2 U/ μ L	50 μ L
Nuclease-free water			9.71 mL

Nuclei Wash Buffer

Reagents	Stock Concentration	Final Concentration	Volumes for 10 mL
PBS	10X	1X	1 mL
10% BSA, Fraction V	10%	2%	2 mL
RNase Inhibitor	40 U/ μ L	0.2 U/ μ L	50 μ L
Nuclease-free water			6.95 mL

Procedure



Critical: All steps must be performed on ice or at 4°C.

1. Add ice-cold lysis buffer to the dissected tissue.
2. Triturate gently to lyse open the cells by pipetting up and down with either a fire-polished silanized Pasteur pipette or a regular-bore pipette tip to break the tissue into small pieces.
3. Centrifuge lysate at 500 x g for 5 min at 4°C to pellet the nuclei.
4. Use a pipette to carefully remove the supernatant.
5. Resuspend the nuclei pellet in 1 mL of ice-cold Nuclei Wash buffer.
6. Repeat wash 3x, centrifuging each time at 500 x g for 5 min at 4°C.
7. Pass nuclei through a 20 μ m cell strainer to filter debris and cell aggregates.
8. Repeat the straining step again, for a total of 2 strains.
9. Count the nuclei.



Note: In our experience, nuclei are much stickier than whole cells.

For researchers making the single-nuclei suspension for the first time, we suggest confirming that the sample contains mostly single nuclei. To do this, take 10 μ L of the sample and stain it with Hoechst, DAPI, or AOPI for more than 5 min, place it on a hemocytometer, and visualize it under a 20X and 40X objective on an epifluorescence microscope. A best practices resource for hemocytometer use, see: <https://www.hemocytometer.org/>

10. Proceed to the appropriate Parse Biosciences Nuclei Fixation Kit [User Guide](#) (either standard NucleiFixation, Low-Input Nuclei Fixation).

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