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Staining Murine Whole Lenses with SynaptoRed™ C2 V.2

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We use this protocol and it's working

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Abstract

Visualization of cellular morphology in intact lenses is essential for studying tissue architecture and cytoskeletal organization. Here, we describe a protocol for fluorescent staining of whole murine lenses using SynaptoRed TM C2 and Hoechst to label plasma membranes and nuclei, respectively. Following *ex vivo* incubation in Medium 199, intact murine lenses were briefly stained with SynaptoRed TM C2 and Hoechst, washed, and imaged immediately using high-resolution confocal microscopy. This staining approach allows clear visualization of cellular boundaries and nuclear organization within the intact lens without the need for fixation or sectioning. Overall, this protocol provides a rapid and reliable method for assessing cell morphology and spatial organization in whole lens tissue.

Materials

SynaptoRed™ C2, Hoechst (Biotium), PBS, medium 199

Stain Preparation

1 SynaptoRed™ C2 stock was made to 10 mM, intermediate stock of 0.1mM (1:100), working solution is 4µM (1:25 dilution of intermediate) in 1x PBS. Hoescht (Biotium) was diluted 1:500 and added to the working stain.

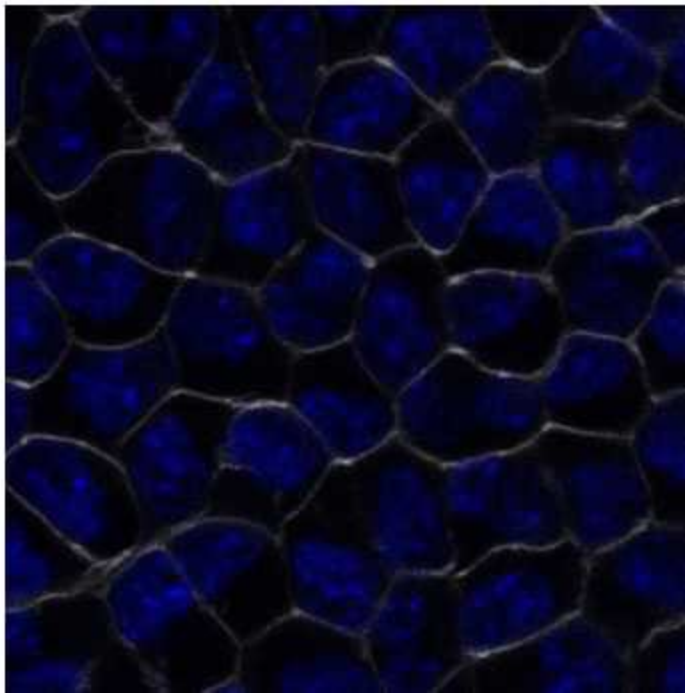
Stain Protocol

2 Murine lens cells were incubated for 2 days at 37°C in 500µL medium 199.

3 After 2 days, lenses were stained with SynaptoRed™ C2 (1:25, final concentration=4µM) and Hoescht (1:500, 1µL) in 480 µL medium 199 for 5 minutes at room temperature.

4 Cells were then washed with PBS and imaged immediately in a divot with a 63x oil objective as previously described (1) with 488 nm excitation/598-758 nm emission for SynaptoRed™ C2 (grey) and 405 nm excitation/410-546 nm emission for Hoescht (blue).

5 Whole murine lens cells stained with SynaptoRed™ C2 and Hoescht to visualize membranes and nuclei, respectively.



Guidelines and Warnings

6 Tissue collection requires prior approval by the user's Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Protocol references

1. Emin G, Islam ST, King RE, Fowler VM, Cheng C, Parreno J. Whole Mount Imaging to Visualize and Quantify Peripheral Lens Structure, Cell Morphology, and Organization. J Vis Exp. 2024 Jan 19;(203):10.3791/66017. doi: 10.3791/66017. PMID: 38314859; PMCID: PMC11623403.