

## Anti-RBPMS Immunofluorescence Protocol

---

**Catalog #:** 1832-RBPMS

**Species:** Guinea Pig

**Tissue:** Retina from adult albino Sprague Dawley rats

---

**Fixation:** 10% Neutral buffered formalin for 1 hour

**Antigen Retrieval:** None

**Antibody incubation:** Primary Antibody- 4C, overnight  
Secondary Antibody- RT, 3 hours or 4C, overnight

---

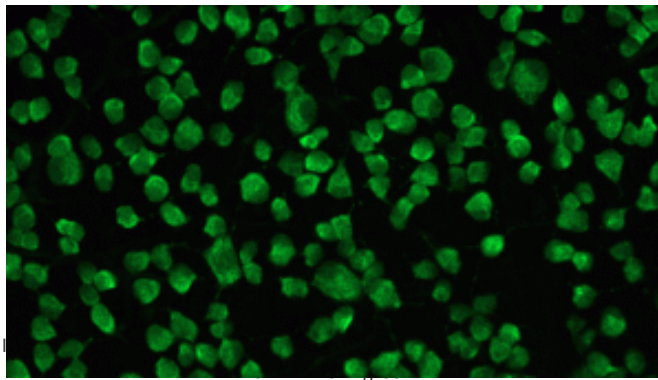
### Materials Required:

- ✓ **Fixative:** 10% Neutral buffered formalin
  - ✓ **1X PBS:** 137 mM NaCl, 28 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.4 mM KCl, 2.9 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.6
  - ✓ **Blocking/Incubation buffer:** TPBS (1X PBS, 0.3% triton x-100), 3% NDS (normal donkey serum) and 1% BSA (Bovine serum albumin)
  - ✓ **Secondary Antibody:** example used is Donkey-Anti-Guinea Pig
  - ✓ **Mounting media:** 50/50 glycerol/1X PBS
  - ✓ **Dissection tools, scalpel, spatula, scissors**
  - ✓ **Rotator**
  - ✓ **Glass slides and cover slip**
- 

### Protocol

1. Remove eye cups containing the retinas from adult albino Sprague Dawley rats and place in 10% neutral buffered formalin under dissecting microscope.
2. Make a small slit in the cornea with a scalpel blade.
3. Use micro dissecting scissors and cut around the cornea starting at the slit.
4. Remove the cornea and take out the lens inside the eye cup.
5. Place eye cup in 5-10 mL of 10% formalin (fixative volume should be at least 20x volume of tissue) and fix for 1 hour at room temperature on a rotator.
6. Rinse with 1X PBS 3 times, 10 minutes each at room temperature.
7. Under dissecting microscope, use micro dissecting scissors to cut around the rim of the eye cup containing the ciliary body.
8. Once the ciliary body is removed, use micro dissecting scissors and cut the optic disc of the retina and ease it out of the eye cup.

9. Place retina in TPBS blocking buffer (1X PBS containing 0.3% triton X-100), 3% NDS, and 1% BSA to block non-specific binding sites for 1 hour at room temperature.
10. After blocking, prepare primary antibody incubation solution with fresh blocking solution and Anti-RBPMS (Catalog# 1832-RBPMS) at a dilution of 1:500. Incubate overnight at 4 C on a rotator.
11. Rinse tissue 3 times with 1X PBS, 10 minutes each.
12. Prepare secondary antibody incubation solution with fresh blocking buffer and secondary antibody (Donkey-Anti-Guinea Pig, Alexa 488, 1:500). Incubate for 3 hours at room temperature or overnight at 4C on a rotator.
13. Rinse 3 times with 1X PBS, 10 minutes each at room temperature
14. Cut the retina into 4 quadrants to flatmount retina in cross like shape in 50:50 glycerol, PBS.



---

*Image and protocol kindly provided by Brian Choi from University of Toronto.*

---