

Anti-RBPMS

Immunohistofluorescence Protocol

Catalog #: 1830-RBPMS

Species: Rabbit

Tissue: Retina from adult C57Bl/6 mice

Fixation: 4% PFA in 1x PBS for 15 minutes then stored in 30% sucrose in 1x PBS for 12-16 hours

Antigen Retrieval: None

Antibody incubation: Primary Antibody- 4C, 5 days in a humidified chamber

Secondary Antibody- 4C, overnight in a humidified chamber

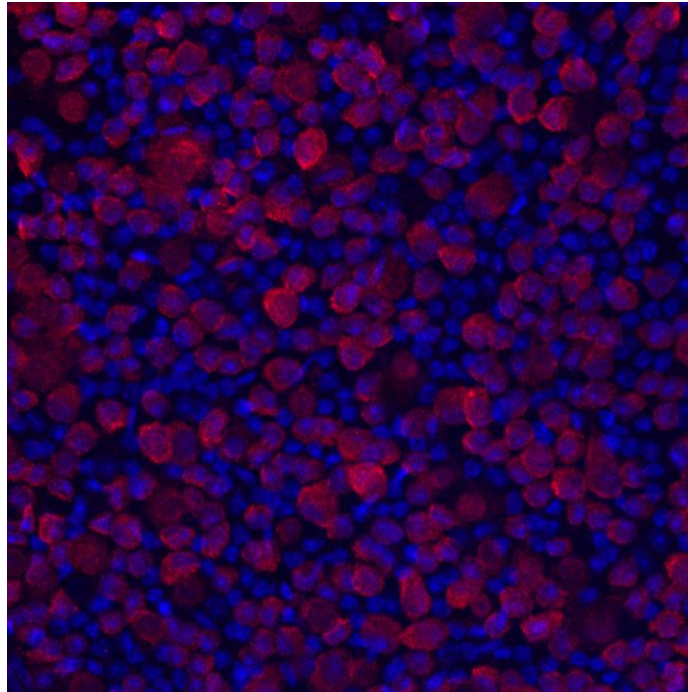
Materials Required:

- ✓ **Fixative(2):** 4% PFA in 1xPBS and 30% sucrose in 1xPBS
 - ✓ **1X PBS:** 137 mM NaCl, 28 mM Na₂HPO₄, 5.4 mM KCl, 2.9 mM KH₂PO₄, pH 7.4
 - ✓ **Blocking Buffer:** 10% normal goat serum (NGS), 1% bovine serum albumin (BSA) and 0.5% Triton X-100 in 1x PBS
 - ✓ **Incubation Buffer:** 3% NGS, 1% BSA, 0.05% sodium azide and 0.5% Triton X-100 in 1xPBS, pH 7.4
 - ✓ **Secondary Antibody:** example used is Alexa 488 Goat-Anti-Rabbit
 - ✓ **Mounting media:** Aqua Poly/Mount (Polysciences, Warrington, PA)
 - ✓ **Dissection tools, scalpel, spatula, scissors**
 - ✓ **Black Filter paper**
 - ✓ **Rotator**
 - ✓ **Glass slides and cover slip**
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Protocol

1. Remove and separate mouse retinas as described in detail by A. Rodriguez, *et al.* 1994. PMID: [24318667](#).
2. Immerse whole mounted retina into 4%PFA in 1xPBS for 15 minutes, then transfer to 30% sucrose in 1xPBS overnight. Both fixative volumes need to be at least 20x volume of the tissue.
3. Rinse with 1X PBS 3 times, 30 minutes each at room temperature.
4. Incubate whole retinas in blocking buffer for 1 hour at room temperature.
5. Rinse with 1x PBS 3 times, 30 minutes each at room temperature.
6. Prepare primary antibody incubation buffer with Anti-RBPMS (Catalog # 1830-RBPMS) at a dilution of 1:1000. Incubate for 5 days at 4C in a humidified chamber.
7. Rinse with 1x PBS 3 times, 30 minutes each at room temperature.

8. Prepare secondary antibody incubation solution with secondary antibody (Goat-Anti-Rabbit, Alexa 647, 1:1000). Incubate overnight at 4C in a humidified chamber.
9. Rinse with 1x PBS 3 times, 30 minutes each at room temperature.
10. Place whole retinas onto a microscope slide, GCL up, and coverslip with Aqua Poly/Mount (Polysciences, Warrington, PA).



Immunostaining of mouse retinal ganglion cells showing specific immunolabeling of RBPMS (1:1000) in red.

Image kindly provided by Allen Rodriguez from the University of California, Los Angeles.

Product and Protocol Specific Reference:

Rodriguez, A.R., de Sevilla Müller, L.P. and Brecha, N.C., 2014. The RNA binding protein RBPMS is a selective marker of ganglion cells in the mammalian retina. *Journal of Comparative Neurology*, 522(6), pp.1411-1443. PMID: [24318667](https://pubmed.ncbi.nlm.nih.gov/24318667/)