

## Cerebellar Immunofluorescence

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v.1

1. Brains need to be perfused with formalin. See perfusion protocol.
2. Prepare 24 well plate with wire screens in wells with 1ml PBS. Only use every other well. Plan wells usage and write on lid to track. Put one to four slices into the wells.
3. Generate 50uM sections of mouse brain on vibratome.  
Sagittal Sections
  - a. Use razor to make a flat sagittal slice  $\frac{1}{4}$  of the way into the brain.
  - b. Make a parallel slice at the midline. Put the intact hemisphere back into the PBS for later.
  - c. Take clean slate block and super glue the brain piece on the block with the vermis (midline) up.
  - d. Put PBS in vibratome well.
  - e. Place block in vibratome with cerebellum away from blade.
  - f. Cut 100uM sections until flat.
  - g. Cut 50uM sections and lift out and put in wells.
4. Unmask epitope by replacing PBS with 0.01M Urea
  - a. 1 M urea stock solution is kept on bench top. Dilute 1:100.
  - b. 3X Heat in microwave for 15 seconds. Cool for 3-10 minutes.
5. Block overnight in 2% goat sera (if secondary is goat), 0.3% triton-X, PBS on rotating rocker at 4°C with rubberbands on wells.  
Add 10ml PBS, 30ul Triton-X, 200ul goat sera
6. Replace block with 1° antibody in block solution. Incubate 4°C on rotator for 48 hours.  
Mouse calbindin 1:500  
Rabbit 11750 1:1000  
Rabbit calbindin 1:1000  
Mouse myc 9E10 1:500
7. Wash 4X in 1ml PBS. Place insert with membranes onto paper towel between washes to wick fluid. On rocker 4°C 20 minute.
8. Wash with 2° antibody 1:500 in block solution. Wrap in aluminum foil incubate 4°C rocker 48 hours.  
Typically Mouse Alexa 488 ( $\approx$ Cy2)  
Rabbit Cy3
9. Wash 4X in 1ml PBS. Place insert with membranes onto paper towel between washes to wick fluid. On rocker 4°C 20 minutes. Keep covered.

## **Mount Slides**

**v.1**

- 1. Turn on heat block to 50°C.**
- 2. Get tubes of NPG (glycerol-gelatin containing 4mg/ml n-propylgalate) and place in heat-block to warm**
- 3. Put 0.5X PBS (1:1 H<sub>2</sub>O:PBS) in p60 plate. Get slides, coverslips, and brushes.**
- 4. Empty membrane out of screen insert into p60**
- 5. Put slide half into p60. Wet face of slide with brush. Slide membrane slice onto slide. Arrange into position and flatten out with brush.**
- 6. Wick fluid off of slide.**
- 7. Put ≈60ul NPG on side of arranged membranes. Lower coverslip over slide.**
- 8. Place on heat-block. Tap if needed to flatten. Make sure NPG is covering membrane slices.**
- 9. Store in dark 20°C in slide holder.**