

Immunohistochemistry for Vibratome Sections of Mouse Brain

I. Intracardiac Perfusion:

- A. Anesthetize the animal with 0.017 ml/g of avertin.
- B. Immobilize the animal on a flat surface and open the thorax: using sharp scissors, cut the skin below the ribs, cut through the diaphragm and up the rib cage lateral to the heart.
- C. Identify the left ventricle of the animal and insert a feeding tube connected to the Fischer Variable Flow peristaltic pump into the ventricle to the ascending aorta. Snip the right atria which allows the blood and perfusion fluid to exit the circulatory system
- D. Perfuse the animal at medium flow rate between 3-5 mls/min (setting 3-4). First purge the brain of blood using 50 ml of ice cold 0.1 M phosphate buffered saline (PBS), pH 7.3-7.4. Then fix the animal using 50 ml 4% paraformaldehyde/PB pH 7.3-7.4. These solutions should be made no more than 24 hours before perfusion and kept cold.
If the lungs begin to inflate or fluid drips from the nose, the perfusion tube is in the right ventricle, not the left. Within the first 2 min. the liver should change from dark red to tan. Check to see if the blood is exiting the right atria.
- E. Block the brain and store in 4% paraformaldehyde overnight at 4 degrees.

II. Tissue Sectioning using a Vibratome (see individual instructions)

- A. Remove the brains from the fixative, blot away excess fluid and secure the brain to the mounting block with *minimal* super glue.
- B. Coating the brain with 2-3% warm agar reduces tissue movement during sectioning and is recommended. Set on ice to cool agar once the brain is coated
- C. Use cold 0.1 M PBS in the vibratome and tissue culture wells to collect tissue sections. Keep the PBS in the vibratome cold by surrounding stage with ice.
- D. Section at 40 μ m. Use a paint brush to transfer the tissue from the vibratome to the wells (24 well dish is fine)

III. Immunohistochemistry

Use a Pasteur pipette that has been bent over a Bunsen burner to transfer the tissue from well to well. Always use a new culture dish (not a washed one) for blocking and antibody incubations

Primary Antibody

- A. Wash the sections 3 times 10 minutes in 0.1 M PBS at room temperature.
- B. Incubate sections in appropriate blocking solution
 - a. If using a mouse monoclonal antibody – follow the M.O.M. kit instructions for blocking
 - b. Otherwise- block in 10% blocking serum/ 0.1% Triton X 100/ 0.1 M PBS at room temperature for 30-40 minutes
- C. Incubate in primary antibody (at appropriate dilution with 3% blocking serum, 0.1% Triton X 100, 0.1 M PBS) overnight at 4 degrees C on a shaker. Cover samples with parafilm (between dish and cover).
- D. When performing double IHC repeat steps A-C using the appropriate block and antibody concentrations

Secondary Antibody Source

Rabbit
Goat/Sheep
Mouse

Blocking Serum

Goat
Rabbit
Horse (or M.O.M. kit)

Secondary Antibody

- A. Wash 3X in 0.1 M PBS for 10 min.each
- B.
 1. If using biotinylated antibody follow Vectastain instructions
 2. If using fluorescence:
 - i. Incubate in block: 10% serum, 0.1% Triton X 100, 0.1 M PBS for 30-40 minutes at room temperature
 - ii. Incubate in appropriate concentration of secondary antibody in 3% serum, 0.1% Triton X 100, 0.1 M PBS 30-40 min. at 4 degrees C on the shaker
When performing double IHC, secondary antibodies can be combined – they must have different sources
- C. Wash 3X in 0.1 M PBS for 10 min. each
- E. Tissue Section Mounting
 - a. Fill a 150 mm petri dish with 0.1 M PBS
 - b. Using a fine paint brush, transfer the sections from the well into the petri dish and float section onto a clean, labeled slide
 - c. Place the slides on a drying block overnight
 - d. Dip the slides *briefly* into tap water to clean off salts
 - e. *Coverslip: use appropriate aqueous mounting media for fluorescence (vectrastain makes one)*