



PRECISIONARY

IMMUNOHISTOCHEMISTRY

BASIC PROTOCOL FOR PROTEIN STAINING IN FIXED TISSUE SLICES

Key to reading the protocol:

√ Rationale for procedural step

♠ Tips & Tricks

1. Obtain cut slices using normal Compresstome® sectioning procedures.
 - √ You can store free-floating sections in PBS or other buffer solutions for about 3-4 weeks at 4° C. Select out the slices you want to stain for IHC, and store the rest until you do more experiments.
2. For [immunohistochemistry](#), first rinse free-floating sections three times in phosphate buffered saline (PBS), 5 min each time.
3. Incubate sections in 10% normal goat serum for non-specific blocking, 1 hour.
4. Incubate sections in primary antibody at 4° C overnight (24 hours).
5. Rinse free-floating sections three times in phosphate buffered saline (PBS), 5 min each time.
6. Include sections in secondary antibody at 25° C (room temperature), 1 hour.
7. Rinse free-floating sections three times in phosphate buffered saline (PBS), 5 min each time.
8. Using a paintbrush, mount sections onto glass slides, then air dry sections.
9. Coverslip slides with mounting media, such as Vectashield mounting media (Vector Laboratories).
 - √ For fluorescent IHC, be sure to cover your slides or keep them in the dark. Do not expose the sections to sunlight, or the protein signal will decrease.

References

* Uses the Compresstome® for for successful immunohistochemistry staining.

1. Abdelaal HM, Kim HO, Wagstaff R, Sawahata R, Southern PJ, Skinner PJ. Comparison of Vibratome and Compresstome sectioning of fresh primate lymphoid and genital tissues for in situ MHC-tetramer and immunofluorescence staining. *Biol Proced Online*. 2015 Jan 7;17(1):2.
2. Duncan J, Kersigo J, Gray B, Fritsch B. Combining lipophilic dye, in situ hybridization, immunohistochemistry, and histology. *J Vis Exp*. 2011 Mar 17;(49).

3. Jameson H, Bateman R, Byrne P, Dyavanapalli J, Wang X, Jain V, Mendelowitz D. Oxytocin neuron activation prevents hypertension that occurs with chronic intermittent hypoxia/hypercapnia in rats. *Am J Physiol Heart Circ Physiol*. 2016 Jun 1;310(11):H1549-57.

4. Selever J, Kong JQ, Arenkiel BR. A rapid approach to high-resolution fluorescence imaging in semi-thick brain slices. *J Vis Exp*. 2011 Jul 26;(53).