



MICROSCOPY

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We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

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An Epitope Retrieval Method For Some Difficult Tissues

Here is an epitope retrieval method that in many cases will allow you to use antibodies in paraffin embedded tissue that could previously only be used in frozen sections, such as PECAM-1, or in tissues that have been over-fixed or over-processed.

Caveat: It is important to note the sequence of events in the protocol, and always use the enzyme treatment first.

- 1) Deparaffinize tissue sections in xylene, 3 X 5 minutes each.

- 2) Rehydrate through graded alcohols to phosphate buffered saline (PBS).
- 3) Place sections in prewarmed (37°C) 0.1% trypsin (Porcine Pancreas, Type II-S) in PBS.
- 4) Rinse in PBS, 3 rinses.
- 5) Place 10 mM citrate buffer, pH 6.0 into a staining dish.
- 6) Microwave for 3 to 4 minutes until a rolling boil.
- 7) Remove from microwave while still boiling, and place slide rack into dish.
- 8) Incubate at room temperature for 15 minutes.
- 9) Rinse in distilled water, then move to PBS.
- 10) Continue with immuno procedure as usual - we put ours into the hydrogen peroxide step next.

We find most antibodies work best with an overnight incubation at 4°C in a humid chamber.

We have used this technique on various antibodies, such as CD8a, CD31, CD34, VEGF, Mac-2, Factor VIII, Bcl-2, Actin, etc. This method also is useful in double labeling immunohistochemical techniques.

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Ultramicrotomy Of Fish Otoliths And Other Hard Tissues

I have been working on the ultrastructure and micro-chemistry of fish otoliths for many years in Hawaii. I have developed a protocol especially for ultramicrotomy of undecalcified otolith. The following is my method:

- 1) Double embedding: Pick up a small piece of otolith (<0.5 mm), embed it with Epon resin. Grind (#600 through #1200 sand paper). As soon as the grinding plane reaches the otolith, stop. Put a drop of Spurr's resin on top and cure it. Then repeat the grinding and embedding 3-5 times. By doing

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