Visualizing Capillary Beds

Standard methods of visualizing capillary beds in tissue often require perfusing the capillaries with colored markers such as India ink or casting materials. The specimen material is subject to substantial artifact due to incomplete perfusion and distortion of delicate vascular components due to excessive perfusion pressure.

A simple method to minimize these potential problems uses the blood components in unperfused tissue. The endogenous peroxidase in red blood cells is easily reduced in the presence of hydrogen peroxide with the resultant oxidation of the chromagen 3,3'-diaminobenzidine (DAB) and formation of a dark reaction product throughout the vasculature. Exposing the tissue sections to peroxidase-conjugated IgG to enhance background staining can further enhance the reaction.

The procedure is as follows:

1) Cool the tissue immediately after euthanizing the animal or removing the organ in order to promote blood clotting.
2) After 1 to 2 hours, cut the tissue into 1 cm³ blocks, immerse in cold 4% paraformaldehyde in 0.1M Phosphate buffer (pH 7.2 to 7.4) and fix for 24 to 48 hours at 40°C.
3) Wash with buffer to remove excess fixative and cut on a vibrating microtome in 50 to 60 µm sections.
4) Immerse sections in a 0.02% (w/v) solution of 3,3'-diaminobenzidine (DAB) containing 0.03% H₂O₂ for 15 to 30 minutes at room temperature.
5) The reaction may be amplified by incubating the tissue sections in a 1:100 (vol/vol) dilution of peroxidase-conjugated goat anti-rat (or appropriate species depending on your tissue). IgG in 0.02 M phosphate buffer (pH 7.2 to 7.4) and fix for 24 to 48 hours at 40°C.

A Blocking Agent for Endogenous Peroxide

We have used 0.1% sodium azide + 3% hydrogen peroxide for many years without damage to acetone-fixed frozen sections. This blocking protocol will work with almost all slide-based specimens for blocking endogenous peroxidase.

The 0.1% sodium azide solution is prepared as a stock solution and the hydrogen peroxide is added prior to use (within several hours). Azide stock is stable for 2 months and a 10X (or 1%) solution can be made if large volumes are used.

The following mix is used for 10 minutes at room temperature after rehydrating slides following fixation in acetone:

- 0.1% sodium azide 45 mL
- 0.3% hydrogen peroxide 5 mL

(Due to 3% hydrogen peroxide for peripheral blood smears)

Discard azide solutions according to waste management practice at your institution.


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To Obtain Vertical Sections of Cell Monolayers for TEM

Our cell monolayers were positioned on the very edge of our specimen blocks. Upon sectioning and mounting, the ultra-thin sections folded over, hampering TEM examination of the cell monolayers. The following soda-straw method centered the monolayers on the specimen blocks and solved our problem:

1) Embed the monolayer within the culture dish with Polybed 812.
2) Following heat polymerization, use a hammer to extract the sample from the dish.
3) Secure the disk-shaped sample in a vise and with a jeweler's or coping...