

Staining protocol for Zinc-fixed mouse tissues

Fix 6-9 hours in Zinc-based fixative: 2.8mM calcium acetate, 22.8mM zinc acetate, and 36.7mM zinc chloride in 0.1M Tris.

Process tissues: dehydrate tissues in 70% EtOH overnight, 100% 3x 45min, Xylene 3X 45 min., paraffin 2x1h

To stain:

Deparaffinize in xylene 2 x 5 min

Rehydrate in 2 x 5 min 100%

Quench in 3% H_2O_2 /methanol 15 min

Rinse 3 x 5 min PBS

Incubate with CD31 1:2000 (biotinylated; Pharmingen #553371) overnight 4°C

Rinse 3 x 5 min PBS

Incubate with ABC 30 min RT

3 x 5 min PBS

Develop with DAB (or HRP chromogen of choice)

Counterstain with methyl green

Dehydrate in 70% 10-15s, 95% 10s, 2 x 100% (long enough to rinse excess methyl green off slides), and xylene 2x 5 min

Coverslip