

Procedure for Recovering Biocytin Stained Cells.
Manis Lab 9/1/89

Adapted from various sources, including Rick Hyson, Dept. Psychol. FSU.

Fixation: in 5% phosphate buffered formalin with 5% dextrose (Grace and Llinas, 1985), pH 7.4. >>> Fix at least overnight.

Cryoprotect:

Wash in PBS, then place in 30% sucrose in PBS overnight. Wash in PBS. Section 30-60 um frozen (in OCT), store in PBS.

Quench endogenous peroxidase:

Incubate for 30 minutes in neat methanol with 0.3% hydrogen peroxide. (Instructions from Vector Kit) (perform in dark or with light shield). Then wash in PBS for at least 20 minutes.

React with avidin-HRP:

Prepare a solution of 1% Triton-X100 in PBS. (1 ml PBS: 10 ul Triton). Add 10 ul Avidin-HRP (corresponds to 1:100 dilution). Mix thoroughly. Incubate slices in this solution for 1-4 hours on rotary shaker.

>>> Rinse slices thoroughly in PBS (3 rinses of 20 minutes each).

Prepare diaminobenzidine solution (DAB):

DAB 0.06% in 0.15M Tris Buffer pH 7.4. DAB stock is 25 mg/ml frozen. Thaw, then mix 25 ul DAB stock in 1 ml Tris buffer. Refreeze DAB stock.

Add 3 ul/ml of 2.5% Cobalt chloride solution stock (1 M stock).

[DAB: 25ul 25mg/ml stock, 3 ul 2.5% cobalt stock, into 1 ml Tris buffer.]

Incubate sections in this medium for 20 minutes with light shield. Sections may develop slight color.

While sections incubating, make 1:100 dilution of fresh H₂O₂ in distilled water (10 ul 30% H₂O₂ in 1 ml dist H₂O).

At end of 20 minute incubation, add 26 ul of this 0.3% Hydrogen peroxide diluted stock to 0.5 ml solution with tissue. Always keep 30% H₂O₂ in refrigerator. Throw away excess diluted peroxide (it doesn't keep).

[H₂O₂: 26 ul/0.5ml well]

Incubate sections with H₂O₂/DAB mixture for 20 minutes more.

Rinse in PBS 3 X 10 minutes each.

Clear in DMSO and view as wet mounts OR :

Mount on subbed slides from PBS. Allow slides to dry at room temperature in dust-free area

overnight (after sections are on slides, blot excess solution and stand slides vertically to dry).

Clear in Xylene 2 minutes.

Coverslip with permount or Fluoromount.