Neurobiotin/biocytin

Making Neurobiotin/biocytin for sharp electrodes

4% Neurobiotin (Vector) in 1 M filtered potassium acetate =
4 mg Neurobiotin in 100 µl 1 M filtered potassium acetate
use 0.2µm filter for potassium acetate

1.5% biocytin =
1.5 mg biocytin in 100 µl 1 M filtered potassium acetate

Injecting Neurobiotin/biocytin into a cell (sharp electrodes)

Use 20 msec pulses of depolarizing current (0.3-5 nA) at frequency that is fast but allows recovery from the pulse to holding potential and about 10 msec longer. So for a 20 msec pulse use a duty cycle of 20 msec on 10-20 msec off. After 2 min hyperpolarizing the cell about 0.5 nA for about 30 seconds. Then repeat.

For electron microscopy, 5 min of dye injection (not including hyperpolarizing 'break' periods) is adequate.

For light microscopy, 10 min is adequate. To fill the axon, 15 min is recommended.

There is variability - some cells fill remarkably fast and even 5 min fills the axon well.

There is not a good correlation between the health of the cell and how well it fills with dye. However, it is rare that a great cell does not fill.

Try to sample a cell at least 50 um deep because cells at the surface can be lost during the processing steps.

Try to sample a cell that does not lie below a cell that was impaled earlier, because both cells may fill.

Neurobiotin/biocytin Processing

1. Incubate sections in 0.5% Triton in 0.1 M Tris buffer (see Stock Solutions for all buffers) overnight.
or incubate in 0.5% Triton for 1 hr
    0.5% = 5 ml 10% Triton in 95 ml 0.1 M Tris buffer

2. Wash sections 3x10 in TRIS A

3. Incubate 30 min in 10% methanol in (3% H_20_2 in TRIS A)
This must be made up immediately before the incubation; it can not be made before hand.
Monitor sections for bubbling; if there is a lot, stop and remake the solution because it was made wrong and the slices could disintegrate if left any longer!
    (3% H_20_2 in TRIS A)= 5 ml 30% in 45 ml TRIS A
    10% methanol in (3% H_20_2 in TRIS A) = 5 ml methanol in 45 ml (3% H_20_2 in TRIS A)

4. Wash in 0.1 M Tris buffer A 3 x 10 min

Optional: Wash in 0.1 M Tris buffer B 10 min

5. Incubate in ABC standard kit (Vector) for at least 2 hr
    5 drops A and 5 drops B in 30 ml TRIS is what you use for this step
    Note phosphate buffer doesn't work

6. Preincubate in DAB

50 mg DAB in 100 ml 0.1 M Tris buffer

Use 50 ml of this and add 20 mg $NiNH_3SO_4$; incubate for 20 min; the $NiNH_3SO_4$ needs to be crushed manually to dissolve. You can do this easily by taking a flat spatula and pressing it against each granule on the side of a tripour beaker.

7. Incubate in DAB

Use the other 50 ml of DAB-TRIS

Add 12.5ul of 30% H_20_2

Transfer sections from the DAB-TRIS-Ni directly to the DAB-TRIS-H_20_2 solution

8. Put sections into 0.1 M Tris buffer to stop the reaction.

9. Let dry after mounting. Let dry at least 8 hrs.

10. Dehydrate in 70% EtOH (10 min) then 90%%, and finally 100%, then place in xylene and coverslip in Permount.

Or

Wash in glycerol, mount in glycerol

Or

Darken further by dipping in 1% osmium. Oamiun is particularly good for axons

Wash in 0.1 M Tris buffer