

VECTASTAIN ABC IMMUNOLABELING OF TEASED SCIATIC NERVES

Description: This protocol is for staining teased peripheral nerve with an antibody for light and EM using Vectastain's ABC Elite kit.

1 AFTER TEASING

- (a) Rinse nerves in 0.1M PBS 2 x 5 minutes on ice.
- (b) Rinse 1x in 0.1M PBS with 50mM glycine for 5 minutes.
- (c) Pretreat nerves in PBS with 0.05-0.1% Triton X-100 and 2% normal serum (NS) plus 0.5% cold water fish gelatin for 20 minutes.
- (d) Incubate nerves in primary antibody diluted in above solution overnight at 4°C.

2 SECONDARY ANTIBODY SOLUTION

- (a) After overnight incubation , wash nerves in PBS 6 x 5 minutes with 0.5% NS (working buffer).
- (b) While washing, make secondary antibody solution: Vectastain biotinylated IgG diluted 1 drop in 10 ml in working buffer plus 0.05% Triton X-100.
- (c) Incubate tissue in the secondary antibody solution for 1 hour on ice.

3 TERTIARY ANTIBODY SOLUTION

- (a) Make tertiary solution using the "A" and "B" solutions from the Vectastain kit: Use 2 drops of "A" and 2 drops of "B" in 5 ml PBS with 0.05% Triton X-100 in working buffer. This solution must be mixed and then allowed to sit for 30 minutes prior to using. While waiting, wash sections 6 x 5 minutes.
- (b) Incubate tissue in tertiary antibody solution for 1 hour on ice.

4 PEROXIDE-DAB SOLUTION

- (a) Wash tissue 6 x 5 minutes with working buffer .
- (b) Make 0.5mg/ml DAB (Sigma) in PBS with H₂O₂ (1-5µL/10 ml).
- (c) Place tissue in DAB/H₂O₂ solution (on ice). The reaction time varies (2-8 min).
- (d) Wash sections in working buffer 3 x 5 minutes

(e) Wash sections in PBS 3 x 5 minutes.

5 POST FIXATION AND EMBEDDING

(a) Fix in 1% OsO₄ in 0.1 M PBS for 30 minutes on ice.

(b) Rinse in DDW 3 x 3 min.

(c) Dehydrate in ethanol series 20, 50, 70, 95, 100, 100% 5 minutes each.

(d) Infiltrate with Durcupan ACM resin and ethanol (1:1) for 1 hour.

(e) Infiltrate with 100% Durcupan resin 1 hour then into fresh 100% 2 hours or overnight.

(f) Polymerize at 60°C between two mold release compound treated glass slides for 24 hr.