SUPPLEMENTAL PROTOCOL

Suggested Protocol for Peroxidase Localization of Wheat Germ Agglutinin in Neural Tissue

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1. Anti-wheat germ agglutinin (made in goat, Cat. No. AS-2024) (approximately 1:500 dilution in 10 mM phosphate, 2.9% NaCl, pH 7.6, 0.1% NaN3, 0.3% Triton X-100 - "Buffer A"). Incubate sections 2 h at 37 °C, then 12–24 h at 4 °C.
2. Rinse 4 x 10 min in buffer A.
3. Biotinylated anti-goat IgG (Cat. No. BA-5000) diluted 1:200 in buffer A. Incubate 2–4 h at room temperature.
4. Rinse 4 x 10 min in buffer A (except no azide).
5. VECTASTAIN® ABC-HRP reagent (Cat. No. PK-4000) or VECTASTAIN® Elite ABC-HRP (Cat. No. PK-6100) made according to package instructions in buffer B (100 mM sodium phosphate-NH4OH buffer, pH 7.0). Incubate 2–4 h (2 h usually sufficient).
6. Rinse 3–4 x 10 min in buffer B.
7. Incubate sections in substrate solution* approximately 10 min.

* Diaminobenzidine (DAB)/hydrogen peroxide is generally used. Two other chromogens can also be used for permanently mounted sections, Vector® VIP and Vector® SG substrate systems. DAB Substrate kit (Cat. No. SK-4100) produces a reddish-brown precipitate; Vector® VIP (Cat. No. SK-4600) produces a violet precipitate; Vector® SG (Cat. No. SK-4700) produces a blue-gray precipitate.

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