SUGGESTED PROTOCOL FOR PEROXIDASE LOCALIZATION OF WHEAT GERM AGGLUTININ IN NEURAL TISSUE

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 X100 - "Buffer A"). Incubate sections 2 hours at 37 °C, then 12-24 hours. at 4 °C.

2. Rinse 4 X 10 minutes in buffer A.

3. Biotinylated anti-goat IgG (Cat. No. BA-5000) diluted 1:200 in buffer A. Incubate 2-4 hours at room temperature.

4. Rinse 4 X 10 minutes in buffer A (except no azide).

5. VECTASTAIN® ABC reagent (Cat. No. PK-4000) or VECTASTAIN® Elite ABC (Cat. No. PK-6100) made according to package instructions in buffer B (100 mM sodium phosphate-NH4OH buffer, pH 7.0). Incubate 2-4 hours (2 hours. usually sufficient).

6. Rinse in buffer B 3-4 X 10 minutes.

7. Incubate sections in substrate solution* approximately 10 minutes.

*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.

Alternate Method

Replace steps 1-3 with the following:

Biotinylated anti-wheat germ agglutinin (Cat. No. BA-0024) 5 µg/ml in buffer A. Incubate 2 hours. at 37 °C and then overnight at 4 °C.

Follow steps 4 through 7 as above.

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