

RABBIT ANTI-SUBSTANCE P POLYCLONAL ANTIBODY

CATALOG NUMBER: AB962

LOT NUMBER:

QUANTITY: 100 μ L

SPECIFICITY: The antiserum has been evaluated for its ability to stain nerve fibers in the external median eminence of the chicken and opossum using both the immunoperoxidase and immunofluorescence procedures.

IMMUNOGEN: Synthetic substance P bound to keyhole limpet hemocyanin (KLH) with carbodiimide.

TISSUE EVALUATION: Formalin-fixed and paraffin-embedded sections (6 microns thick) of median eminence tissue were prepared from the domestic chicken and opossum. Cryostat sections of the above tissues can also be used in the staining procedure.

IMMUNOPEROXIDASE STAINING:

1. The sections to be stained are hydrated to phosphated buffered saline (PBS, pH 7.4).
2. The antiserum diluted 1:500-1:1,000 (in PBS) is allowed to react with the tissue at 37°C for 30 minutes.
3. Using PBS, the tissue is washed for 15 minutes at room temperature and treated sequentially with goat anti-rabbit gamma globulin (diluted 1:20 in PBS) and peroxidase anti-peroxidase complex (diluted 1:100 in PBS) for 30 minutes at 37°C. After each of these two steps excess reagent is washed from the section as described before.
4. The final step in the staining involved treating section with a mixture of 3,3'-diaminobenzidine HCl (0.02%) in hydrogen peroxide (0.004%). For preservation, the sections are dehydrated, cleared and mounted.

IMMUNOFLUORESCENCE STAINING:

1. Same as Step 1 above.
2. The tissue is reacted with the antiserum, diluted 1:20-1:40 in (PBS) for 30 minutes at 37°C.
3. After washing off the excess antiserum with PBS, the section is treated with fluorescein-labeled goat anti-rabbit gamma globulin serum (diluted 1:10 in PBS) for 30 minutes at 37°C. The tissue is washed with PBS and after mounting under saline-glycerol, is viewed with a fluorescence microscope.

COMMENTS: The antiserum described here has been established to give a positive staining of the nerve fibers in the median eminence of two species using the above protocol. It is recommended, however, that the customer establishes his own optimal dilutions to give positive staining. In general, staining can occur on further dilution of the primary antiserum if this is accompanied by an increase in its incubation time, e.g. 24 hours.

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- PRESENTATION:** Liquid antiserum. Contains 0.1% sodium azide.
- STORAGE/HANDLING:** Store at -20°C in undiluted aliquots for up to 12 months after date of receipt. Avoid repeated freeze/thaw cycles.
- REFERENCES:**
1. Nairn, R. C. *Fluorescent protein tracing*. Livingstone, Edinburgh, (1964).
 2. Sternberger, L. A. *Immunocytochemistry*. Wiley, New York (1979).
 3. Ho, R. H., et al. *Brain Res.*, **189**:565-569 (1980).
 4. Hokfelt, T., et al. *Proc. Natl. Acad. Sci.*, **75**:1013-1015 (1978).
 5. Hokfelt, T., et al. *Neuroscience*, **3**:17-538 (1978).
 6. Hokfelt, T., et al. *Brain Res.*, **100**:235-252 (1975).
 7. Cheung, W.M.W., et al., *BioTechniques* (1999) **26**:946-954.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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