



RABBIT ANTI-LEU-ENKEPHALIN POLYCLONAL ANTIBODY

CATALOG NO:	AB5024
LOT NUMBER:	
QUANTITY:	50 µg
CONCENTRATION:	1 mg/mL
SPECIFICITY:	Leu ⁵ -enkephalin. Cross reactivity: Met ⁵ -enkephalin 0.93%, beta-endorphin <0.1% and bovine beta-Lipotropin <0.1%.
IMMUNOGEN:	Synthetic Leu ⁵ -enkephalin peptide (H-Try-Gly-Gly-Phe-Leu-OH) conjugated through the N-terminal tyrosine to BSA.
APPLICATIONS:	Immunohistochemistry: 2-10 µg/mL. See suggested protocols. ELISA/RIA: 2-10 µg/mL Optimal working dilutions must be determined by the end user.
SPECIES REACTIVITIES:	Human, rat and mouse.
FORMAT:	Purified immunoglobulin.
PRESENTATION:	Liquid. Contains 0.1% sodium azide as a preservative.
STORAGE/HANDLING:	Maintain material at -20°C in undiluted aliquots for up to six months after date of receipt. Avoid repeated freeze/thaw cycles.
REFERENCES:	1) <i>Neuroscience</i> (1976) 1 :349-351. 2) <i>Histochemistry</i> (1981) 73 :89-114. 3) <i>Progress in Neurobiology</i> (1984) 23 :151-189. 4) <i>Neuroscience & Biobehavioral Reviews</i> (1985) 9 :573-587. 5) <i>Histochemistry</i> (1985) 83 :47-56. 6) <i>Neurosurgical Review</i> (1991) 14 :97-110.



APPLICATION NOTES FOR AB5024

IMMUNOHISTOCHEMISTRY

Anesthetize animals and perfuse them transcardially as follows:

- Flush with cold (4°C) oxygenated Calcium free Tyrodes
- Perfuse with 4% formaldehyde in 0.16M phosphate buffer at pH 6.9
- Perfuse with 10% sucrose solution in 0.1M phosphate buffer (pH 7.2)

Sections

Cut 5-30 μm sections using a cryostat and mount them on subbed histological slides.

Immunofluorescence

Dilute Leu-enkephalin antibody with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% BSA and 0.01% Triton X-100. Incubate sections overnight at 2-8°C and then wash in PBS (three times for 10 minutes). Incubate for one hour at room temperature with properly diluted donkey anti-rabbit secondary antibody conjugated to a fluorescent probe such as FITC (Catalog Number AP182F), Rhodamine (Catalog Number AB182R) or Cy3 (Catalog Number AP182C). Wash in PBS (three times for 10 minutes) and mount with a PBS/glycerol solution containing 0.1% phenylenediamine to reduce fading. If not stained with FITC, sections can be dehydrated in grading alcohols (50%, 75%, 80%, 96% and 100%), cleared with xylene and mounted with DPX (Fluka, Ronkonkoma, NY). Staining can be visualized by using both conventional and confocal microscopy.

Indirect Avidin-Biotin Peroxidase

Incubate sections with 0.3% H_2O_2 in PBS for 15 minutes at room temperature to block endogenous peroxidase. Rinse sections with PBS (three times for 10 minutes). Dilute Leu-enkephalin antibody with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% BSA and 0.01% Triton X-100. Incubate sections overnight at 2-8°C and then wash in PBS (three times for 10 minutes). Incubate sections with properly diluted biotinylated goat anti-rabbit secondary antibody (Catalog Number AP132B) diluted in PBS (do not add sodium azide!) for one hour at room temperature. Rinse sections three times for 15 minutes and incubate sections with horseradish peroxidase-streptavidin complex (Catalog Number SA108) properly diluted in PBS/0.1% Triton X-100 for 40 minutes at room temperature. Wash sections three times for 15 minutes in PBS and incubate them with 0.03% of diaminobenzidine (DAB) in PBS/0.015% H_2O_2 for 3-15 minutes at room temperature. Dehydrate sections in grading alcohols (50%, 75%, 80%, 96% and 100%), clean them in xylene and coverslip with DPX or Permount. Sections can be examined using conventional microscopy.

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 μL 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.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC
PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2002 - 2010: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com