



RABBIT ANTI-ATRIAL NATRIURETIC PEPTIDE AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER:	AB5490
LOT NUMBER:	
QUANTITY:	100 µg
CONCENTRATION:	1 mg/mL
SPECIFICITY:	Atrial Natriuretic Peptide (ANP).
IMMUNOGEN:	Rat ANP conjugated to BSA.
APPLICATIONS:	Immunohistochemistry: 2-10 µg/mL. See suggested protocol. It is expected that the antibody will also work for Western blot, ELISA and RIA. Optimal working dilutions must be determined by the end user.
SPECIES REACTIVITIES:	Human, rat and mouse.
FORMAT:	Affinity purified immunoglobulin.
PRESENTATION:	Liquid containing 0.1% sodium azide.
STORAGE/HANDLING:	Maintain at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles.
REFERENCE:	Kehat, I., et al., Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiocytes. <i>The Journal of Clinical Investigation</i> . (2001) 108 :407-414.
RELATED REFERENCES:	<ol style="list-style-type: none">1. Cho, EE, et al., <i>Arch Oral Biol</i> (2000) 45:425-430.2. Ohmori, Y, <i>Horm Metab Res</i> (1998) 30:384-388.3. Maley, BE, <i>Chem Senses</i> (1996) 21:367-376.4. Jougasaki, M, et al., <i>Am J Physiol</i> (1995) 268:F657-663.5. McKenzie, JC, <i>J. Histochem Cytochem.</i> (1992) 40:1211-1222.6. Kawaguchi, S, et al., <i>J. Histochem Cytochem.</i> (1989) 37:1739-1742.7. Yamada, H, et al., <i>Histochemistry</i> (1988) 89:411-413.8. Chapeau, C, et al., <i>J. Histochem Cytochem.</i> (1985) 33:541-550.9. Cantin, M, et al., <i>Histochemistry</i> (1984) 80:113-127.

APPLICATION NOTES FOR AB5490

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 μ m sections using a cryostat and mount them on subbed histological slides.

Immunofluorescence

Dilute ANP antibody with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% BSA and 0.1% Triton X-100. Incubate sections for 24-48 hours at 2-8°C. Wash in PBS (three times for 10 minutes). Incubate for one hour or two hours 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% H₂O₂ in PBS for 15 minutes at room temperature to block endogenous peroxidase. Rinse sections with PBS (three times for 10 minutes). Dilute ANP antibody with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% BSA and 0.1% 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 PBS and incubate them in substrate solutions (e.g. DAB, AEC, etc.) to achieve necessary intensity of ANP staining.

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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PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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