

MVS Pacific, LLC
P.O. Box 14962
Minneapolis, MN 55414
www.mvspacific.com

Antiserum Data Sheet

Warning: This product is intended for research or manufacturing use only. It is pharmaceutically unrefined and verification of its solvency for use in humans or as a clinical diagnostic reagent and the compliance with all Federal and State laws regulating such applications is the sole responsibility of the purchaser.

Immunogen

Antibodies were affinity purified from the antiserum raised in *rabbits* against the rat atrial natriuretic peptide (ANP) conjugated to BSA.

Cat # AD 35-005

Applications

Immunocytochemistry, IP, RIA

Storage and Reconstitution

This product is a lyophilized antiserum. It is recommended to store this antiserum at 0-4°C prior to reconstitution. Antiserum may be reconstituted with PBS (to get final concentration of 1 mg/ml) containing 0.1% of sodium azide or other preservative. For prolonged storage divide antiserum into aliquots and store frozen at -20°C or lower. Avoid repeated freezing and thawing. Frozen aliquotes can be stored for at least six months. For better performance, once a frozen aliquot has been thawed, make a working dilution (antibody dilution buffer should contain 0.1% of sodium azide or other preservative) and keep it at 0-4°C (stable for at least 1 month).

Optimum working dilution for immunofluorescence histochemistry is from 10-15 ug/ml. However, depending on tissue fixation procedures and type of immunocytochemistry protocols (free floating sections vs. slide-mounted sections), working dilutions should be determined by individual investigator.

Known species' cross-reactivity

Rat, mouse

Exemplary Immunocytochemical Protocol

Anesthetize animals and perfuse them transcardially as follows:

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

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

Immunofluorescence: Dilute Leu⁵-Enkephalin antiserum with 0.1M phosphate buffered saline (PBS, pH 7.4) containing 1% bovine serum albumin and 0.01 Triton X-100. Incubate sections overnight at +4°C and then wash in PBS (three times for 10 minutes). Incubate for 1 hour at room temperature with donkey anti-rabbit secondary antibodies conjugated to fluorescent probes such as fluorescein isothiocyanate (FITC), Lissamine rhodamine sulfonyl chloride (LRSC); cyanine 3.18 (Cy3) or cyanine 5.18 (Cy5) (for reference see a catalogue of Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Wash in PBS (three times for 10 minutes) and mount them 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 in xylene and mounted with DPX (for reference see a catalogue of Fluka, Ronkonkoma, NY). Staining can be visualized by using both conventional and confocal microscopy.

Indirect immunostaining technique: 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), incubate sections overnight at +4°C and then wash in PBS (three times for 10 minutes). Incubate sections with biotinylated goat anti-rabbit secondary antibodies diluted 1:300 in PBS (do not add sodium azide!) for 1 hour at room temperature, rinse sections three times for 15 minutes and incubate sections with horseradish peroxidase-streptavidin complex 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 in 0.03% of diaminobenzidine (DAB) in PBS/0.015% H₂O₂ for 3-15 minutes at room temperature. Dehydrate sections in grading alcohols (50%, 75%, 80%, 96% and 100%), clear them in xylene and coverslip with DPX or Permount. Sections can be examined by using conventional microscopy.

References

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