MVS

# AD-32-005

*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 antiserum raised in *rabbits* against synthetic Somatostatin ($NH_2$-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-COOH) conjugated to BSA.

**Cat #** AD 32-005

**Applications**
Immunocytochemistry, WB, 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 5 - 10 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, human

**Immunocytochemical Protocol**
Anesthetize animals and perfuse them transcardially as follows:

a. flush with cold (4°C) oxygenated Calcium free Tyrodes;
b. perfuse with 4% formaldehyde in 0.16 M phosphate buffer at pH 6.9.
c. 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 $Leu^5$-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% $H_2O_2$ 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 biotinilated 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 horseraddish 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% $H_2O_2$ 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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