



Leu-Enkephalin

Data Sheet

Catalog Number:	RA21007	Host:	Rabbit
Product Type:	Affinity Purified Antibody	Species Reactivity:	Rat
Immunogen Sequence:	Synthetic Leu ⁵ -enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) conjugated through N-terminal tyrosine to BSA.	Format:	Liquid -PBS with 0.1% Sodium Azide. Concentration of 1 mg/ml.
Applications:	For immunofluorescence cytochemistry 1:1000 For indirect avidin-biotin immunocytochemistry 1:1000 - 1:10,000.		
Storage:	*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.		

Application Notes

Sensitivity

10% inhibition of binding (determined by RIA) is 3-5 pg
IC₅₀ 30-50 pg.

Cross-reactivity (%)

Leu ⁵ -Enkephalin	100
Met ⁵ -Enkephalin	0.93
beta-Endorphin	0.01
beta-Lipotropin	0.01

Immunofluorescence 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.

- 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), Rhodamine Red X; 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 using Neuromics iBrite Plus anti-fade mounting medium (cat#: SF40000). Staining can be visualized by using both conventional and confocal microscopy.

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References

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6. Moore MR, Black PM. (1991) *Neurosurgical Review*, 14(2):97-110.

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