

## Product Information

### ANTI- $\beta$ -ENDORPHIN

Developed in Rabbit  
Delipidized, Whole Antiserum

Product Number **E1520**

#### Product Description

Anti- $\beta$ -Endorphin is developed in rabbit using synthetic rat  $\beta$ -endorphin conjugated to KLH as the immunogen. The antiserum has been treated to remove lipoproteins.

Anti- $\beta$ -Endorphin specifically stains pituitary cells in 4% paraformaldehyde perfusion-fixed, frozen section of rat pituitary. Specific staining is inhibited with rat  $\beta$ -endorphin. Anti- $\beta$ -Endorphin reacts in dot blot immunoassay with rat  $\beta$ -endorphin conjugated to BSA. Cross-reactivity is observed with  $\beta$ -endorphin (1-27),  $\alpha$ -endorphin, and  $\gamma$ -endorphin conjugated to BSA. No cross-reactivity is observed with [Leu<sup>5</sup>]-enkephalin and [Met<sup>5</sup>]-enkephalin conjugated to BSA.

Anti- $\beta$ -Endorphin may be used to detect  $\beta$ -endorphin by immunohistochemistry in paraformaldehyde-fixed, frozen tissue sections of CNS and peripheral tissue of various species, and may be used in various immunochemical methods such as RIA and dot blot immunoassay.

Endorphin consists of a family of endogenous opioid peptides originally isolated from the pituitary gland. These peptides, known as  $\alpha$ -,  $\beta$ - and  $\gamma$ -endorphin, have potent opiate and neuroendocrine activities.<sup>1-3</sup> They are formed by cleavage from a common precursor protein  $\beta$ -lipotropin (1-91) [ $\beta$ -LPH (1-91)].<sup>4</sup>  $\beta$ -LPH itself arises along with adrenocorticotrophic hormone (ACTH) and  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH) from a large precursor, proopiomelanocortin (POMC).

$\beta$ -Endorphin, the C-terminal 31 amino acid fragment of  $\beta$ -LPH, [ $\beta$ -LPH(61-91)], is major peptide hormone and neuropeptide in the pituitary and central nervous system (CNS).<sup>1</sup>  $\beta$ -Endorphin is the most potent opiate peptide among the three endorphins. It has high affinity for brain opiate receptors, it elicits analgesia and can produce profound sedation and catatonia *in vivo*.<sup>1</sup>

$\beta$ -Endorphin is highly concentrated in the corticotrophic cells of the anterior pituitary and in the intermediate

lobe of the pituitary.<sup>5</sup> In the CNS,  $\beta$ -endorphin-containing neurons are localized to specific areas of the brain, with their cell bodies highly concentrated in the hypothalamus.<sup>6-8</sup> Neuronal fibers originating from these neurons extend to several regions of the brain.

Antibodies that react specifically with  $\beta$ -endorphin are useful for the study of the mode of action, differential tissue expression, intracellular and subcellular localization of  $\beta$ -endorphin in the CNS and peripheral nervous system.

#### Reagents

The product is provided as whole antiserum containing 0.1% sodium azide (see MSDS)\* as a preservative.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

#### Product Profile

Protein Concentration: Determined by biuret.

#### Immunohistology

A minimum dilution of 1:1,000 was determined by immunohistochemistry using 4% paraformaldehyde perfusion-fixed, frozen sections of rat pituitary. Anti- $\beta$ -Endorphin specifically stains  $\beta$ -endorphin-containing cells in rat pituitary.

Note: The visualization of specific staining is inhibited by pre-incubation of diluted antiserum with  $\beta$ -endorphin. Neuronal cell bodies may require pre-treatment of the animals with inhibitors of axonal transport such as colchicine.

#### Dot Blot

A minimum dilution of 1:8,000 was determined by dot blot immunoassay using rat  $\beta$ -endorphin conjugated to BSA (120 ng/dot).

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

#### RIA Dilution Instructions

A working dilution of 1:100 was determined using 100-200 pg/tube of <sup>125</sup>I-labeled β-endorphin in a second antibody and polyethylene glycol RIA method.

It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in systems and procedures.

#### RIA Specificity

Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in the second antibody-PEG <sup>125</sup>I RIA system is as follows:

Peptide	% Cross-Reactivity at 50% displacement
β-Endorphin (rat)	100
β-Endorphin (human)	100
β-Endorphin (bovine)	≤100
α-Endorphin	≤30
γ-Endorphin	≤20
[Leu <sup>5</sup> ]-enkephalin	≤0.05
[Met <sup>5</sup> ]-enkephalin	≤0.05

#### RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B<sub>0</sub> standard curve. In the above system, the sensitivity has been found to be at least 4 pg/tube of rat β-endorphin.

#### Affinity Constant

The affinity constant (K<sub>a</sub>) is determined by a Scatchard plot using this RIA system.

$$K_a = 1 \cdot 10 \times 10^{11} \text{ L/M}$$

#### Storage

For continuous use, store at 2-8°C. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

#### References

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5. Liotta, A., et al., *Proc. Natl. Acad. Sci. USA*, **75**, 2950 (1978).
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