



## Product Information

### Anti-Substance P (SP) Developed in Rabbit Delipidized, Whole Antiserum

Product No. **S 1542**

Anti-Substance P (SP) is developed in rabbits using synthetic substance P conjugated to KLH as the immunogen. The product is provided as whole antiserum with 0.1% sodium azide (see MSDS)\* as a preservative.

#### Specificity

Anti-Substance P reacts with substance P (SP) conjugated to BSA in dot blot. Cross-reactivity is observed with substance P fragments SP(7-11) and SP(6-11) conjugated to BSA in dot blot. Weak cross-reactivity is observed with SP(1-7) and neurokinin A conjugated to BSA by dot blot. The antiserum shows no cross-reactivity with neurokinin B, neuropeptide Y (porcine), vasoactive intestinal peptide, calcitonin gene related peptide (CGRP) (rat), calcitonin, and somatostatin conjugated to BSA.

#### Description

Substance P (SP), an undecapeptide with the sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub>, is a neuropeptide with a broad spectrum of biological activities<sup>1</sup>. SP exerts potent actions on smooth muscle, glandular tissue and the central nervous system (CNS)<sup>1,2,3</sup>. SP belongs to the tachykinins, a family of peptides that includes neurokinin A (NKA), neurokinin B (NKB) and their extended forms in mammals. SP and NKA are encoded by the same gene, preprotachykinin A (PPT-A), that gives rise to  $\alpha$ ,  $\beta$ , and  $\gamma$ -PPT-A mRNA by alternative splicing.<sup>5,6</sup> SP is released from its precursors by proteolytic cleavage at pairs of basic amino acids residues. Considerable evidence indicates that SP is a neurotransmitter or neuromodulator.<sup>7,8</sup> SP is widely distributed throughout the CNS and peripheral nervous system, including the brain, spinal cord, primary sensory neurons and intrinsic nerve fibers associated with the gastrointestinal (GI) tract, salivary glands, respiratory tract, kidney, urogenital tract, skin, blood vessels and smooth muscle.<sup>1,7,9,10</sup> In primary sensory neurons, SP is mainly co-localized with calcitonin-gene related peptide (CGRP) and neurokinin A (NKA).

Anatomical, neurophysiological and pharmacological evidence indicates a role of SP in the transmission of pain. SP mediates the process of neurogenic inflammation.<sup>11</sup> SP is selectively localized in sensory systems (i.e., small cell bodies of sensory ganglia, fine unmyelinated sensory fibers, dorsal horn of the spinal cord and pain fibers of the trigeminal nucleus). SP has a potent excitatory effect on dorsal horn neurons which respond to certain pain stimuli.<sup>12</sup> The mammalian tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), act on multiple neurokinin receptor subtypes to induce their physiological actions. Three different NK receptors have been identified and cloned, NK1, NK2 and NK3.<sup>13,14</sup> These receptors are encoded by different mRNAs and are differentially expressed in the brain and the peripheral tissue. SP, NKA and NKB preferentially, but not exclusively, bind to the NK1, NK2 and NK3 receptors, respectively. Antibodies that react specifically with SP may be used to study the mode of action, differential tissue expression and intracellular and subcellular localization of SP in the CNS and peripheral nervous system.

#### Uses

Anti-Substance P (SP) may be used for the immunodetection of SP in various immunoassays including dot blot, RIA and ELISA. The antibody localizes SP by various immunohistochemical methods using formalin-fixed, frozen or Vibratome sections of rat, cat, porcine, bovine, monkey and human brain.

**Protein Concentration:** Determined by Biuret.

#### Working Dilution

A minimum working dilution of 1:20,000 was determined by dot blot immunoassay using Substance P-BSA (0.03 - 0.25 ng/dot).

In order to obtain best results in different preparations, it is recommended that each individual user determine their optimal working dilutions by titration assay.

### RIA Dilution Instructions

The minimum working dilution is determined to be 1:5,000 using 10 pg/tube of <sup>125</sup>I-labeled Substance P in a second antibody and polyethylene glycol RIA. 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 I<sup>125</sup> RIA system is as follows:

Cross-Reactant	%Cross-Reactivity
Substance P	100
Substance P (2-11)	400
Substance P (3-11)	122
Substance P (4-11)	175
Substance P (5-11)	281
Substance P (6-11)	400
Substance P (7-11)	0.96
Neurokinin A	0.2
Endothelin 1	0.2
Somatostatin	0.3

### 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 10 pg/tube.

### RIA Affinity Constant

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

$$K_a = 10 \times 10^{10} \text{ L/mole.}$$

### Storage

For continuous use, store at 2-8 °C. For extended storage, solution may be frozen 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 by centrifugation before use.

### Precautions and Disclaimer

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

### References

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