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ProductInformation

Anti-Endothelin
antibody produced in rabbit, whole antiserum

Catalog Number **E1645**

Product Description

Anti-Endothelin is developed in rabbit using as immunogen a synthetic human endothlein-1 conjugated to KLH. The antiserum has been treated to remove lipoproteins.

Anti-Endothelin reacts with human or pig endothelin-1 (ET-1), human endothelin-2 (ET-2), and human or rat endothelin-3 (ET-3). Cross-reactivity is observed with sarafotoxin S6c. Minimal cross-reactivity is observed with angiotensin 1. No cross-reactivity is observed with human big endothelin 38, rat big endothelin 39, or human atrial natriuretic peptide. Applications include the detection of endothelin by radioimmunoassay (RIA) and ELISA.

Endothelin consists of a family of potent vaso-constrictor peptides, which include four structurally-related isoforms, ET-1, ET-2, ET-3, and vasointestinal contractor (VIC, β-ET).[1] Endothelin-1 (ET-1), a 21 amino acid peptide produced by vascular endothelial cells, is a potent vasoconstrictor which plays an important role in the homeostasis of the circulatory system and in pathogenesis of cardiovascular diseases.[1-3] The endothelin isoforms are distinct in their pharmacological activities and distribution, but only ET-1 is synthesized by vascular endothelial cells. The endothelins share remarkable sequence homology and have similar biological activities with a group of peptide toxins from snake venom called sarafotoxins.[4]

Endothelin-1 is formed by proteolytic processing of a larger precursor peptide, big endothelin 39 in pig or big endothelin 38 in humans. The amino acid sequences of mature human and pig ET-1 are identical. In addition to the potent vasoconstrictor and vasopressor actions, ET-1 has a wide range of biological activities in various tissues including contraction of airway and intestinal smooth muscle, release of vasodilator prostaglandins and nitric oxide (NO), mitogenic effects on vascular smooth muscle cells and fibroblasts, stimulation of atrial natriuretic peptide secretion from atrial cardiocytes, and inhibition of renin release.[5-8]

In peripheral tissue and brain, ET-1 is a potent stimulator of inositol phospholipid turnover. In the central nervous system (CNS), ET-1 is widely distributed and may be found in cerebral vascular smooth muscle, neurons, and glia cells. In the CNS, ET-1 is expressed in the spinal cord and dorsal root ganglia, where it may serve as a neurotransmitter/neuromodulator.[9-11] It may serve various functions including central regulation of blood pressure and respiratory functions.

The biological actions of endothelins are mediated by activation of phospholipase C through specific G protein-coupled receptors. Two distinct receptor subtypes, ET_A and ET_B receptors, have been cloned, which have different ligand preference and are differentially distributed in various peripheral tissues and the CNS.[12] Antibodies that react specifically with endothelins (ET-1, ET-2, and ET-3) may be used to detect endothelins in tissue extracts and biological fluids as well as study differential tissue expression, and intracellular localization of the endothelin isoforms in the periphery and central nervous system.

Reagent

The product is supplied as whole antiserum containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2–8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in “frost-free” freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Indirect ELISA: a working antibody dilution of 1:30,000 is recommended using 1.25 ng of endothelin-1/well for coating. In competition assays, the antibody is inhibited by human and pig endothelin-1. Cross reactivity is observed with human endothelin-2, and human or rat endothelin-3. Minimal cross-reactivity is observed with human big endothelin 38 (see Table 1). Competing peptide concentration is 1 nmol/mL to 0.01 pmol/mL.

Table 1.

Specificity by ELISA

Peptide	% Cross-reactivity by ELISA
Endothelin-1 (human, pig)	100
Endothelin-2 (human)	55
Endothelin-3 (human, rat)	55
Big Endothelin 38 (human)	0.1

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

Radioimmunoassay: a working antibody dilution of 1:10,000 is recommended using 5–10 pg/tube of ^{125}I- labeled human endothelin-1 in a second antibody and polyethylene glycol RIA.

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the described system, the sensitivity has been found to be 5 pg of endothelin per tube.

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.

$$K_a = 1.6 \times 10^{10} \text{ L/mole}$$

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 ^{125}I RIA system is as follows:

Peptide	%Cross-Reactivity at 50% displacement
Endothelin-1 (human, pig)	100
Endothelin-2 (human)	100
Endothelin-3 (human, rat)	100
Sarafotoxin S6c	65
Big Endothelin 38 (human)	0.01
Big Endothelin 39 (rat)	0.01
Atrial Natriuretic Peptide (human)	0.01
Angiotensin I (human)	0.1

References

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