SIGMA CHEMICAL COMPANY

Product No. E-1645
Lot 096H4859

Anti-Endothelin

Developed in Rabbit
Delipidized, Whole Antiserum

Anti-Endothelin is developed in rabbit using synthetic human endothelin-1 conjugated to KLH as the immunogen. The antiserum has been treated to remove lipoproteins. The product is provided as whole antiserum with 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

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

Description

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 inositolphospholipid 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.

Uses

Anti-Endothelin may be used for the immunodetection of endothelins (ET-1, ET-2, and ET-3) in various immunoassays including RIA and ELISA.

Protein Concentration: 61.8 mg/ml by biuret.

ELISA

A titer of 1:30,000 was determined by indirect ELISA using 1.25 ng 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 below). Competing peptide concentration is 1 nmol/ml to 0.01 pmol/ml.

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

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 1:10,000 was determined using 5-10 pg/tube of ^{125}I-labelled endothelin-1 in a second antibody and polyethylene glycol RIA.

It is recommended that the antiserum first be evalutated 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^{125} 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

RIA Sensitivity

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

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using this RIA system.
$K_a = 1.6 \times 10^{10}$ L/mole.

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.

***** 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.

References

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12. Sakurai, T., et al., Trends Pharmacol. Sci., **13**, 103 (1991).