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Product Information

MONOCLONAL ANTI -ENDOTHELIN CLONE ET-1/58 Mouse Ascites Fluid

Product No. E 0771

TECHNICAL BULLETIN

Product Description

Monoclonal Anti-Endothelin (mouse IgG2a isotype) is derived from the ET-1/58 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Synthetic human endothelin-1, conjugated to KLH was used as the immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Endothelin consists of a family of potent vasoconstrictor 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 have similar biological activities and share remarkable sequence homology with a group of peptide toxins from snake venom, the sarafotoxins.⁴ Endothelin-1 is formed by proteolytic processing of a larger precursor peptides: porcine big endothelin-39, or human big endothelin-38. The amino acid sequences of mature human and porcine 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 rennin release.⁵⁻⁸

In peripheral tissues and brain, ET-1 is a potent stimulator of inositol-phospholipid turnover. ET-1 is widely distributed in the central nervous system (CNS), and in addition to cerebral vascular smooth muscle, is found in neurons and glia cells. It is expressed in the spinal cord and dorsal root ganglia, where it may serve as a neurotransmitter/neuromodulator.⁹⁻¹¹ In the CNS, ET-1 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, have been cloned, that 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) are useful for the detection of endothelins in tissue extracts and biological fluids and for the study of differential tissue expression and intracellular localization of the endothelin isoforms in the nervous system.

Reagents

Monoclonal Anti-Endothelin is provided as ascites fluid with 0.1% sodium azide 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.

Storage/Stability

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.

Second Antibody Radioimmunoassay Procedure

Reagents required

1. Buffer I: 10 mM PBS, pH 7.2-7.4, with 0.3% BSA, 0.1% Triton, 1 mM EDTA and 0.1% NaN₃.
2. Buffer II: 10 mM ammonium acetate, pH 5.0
3. Buffer III: 10 mM PBS, pH 7.2-7.4 (Product No. P 4417)
4. Dilute Monoclonal Anti-Endothelin to the recommended working dilution, with Buffer I.
5. Radiolabeled tracer: Freshly prepared solution of 5-10 pg/ml ¹²⁵I-labeled-endothelin-1 (human) in Buffer I, using ¹²⁵I-endothelin-1 with specific activity of approx. 2,000 ci/mmmole (approx. 150,000 - 200,000 dpm/ml).
6. Endothelin-1 (Product No. E 7764). Prepare solution of 1 mg/ml in Buffer II. Dilute to 100 ng/ml with Buffer I. Continue with 6 serial dilutions with Buffer I (100-1.56 ng/ml).
7. 0.1 M EDTA, pH adjusted to 7.8.
8. 2% solution of normal mouse serum (Product No. M 5905) in Buffer III.
9. Anti-Mouse IgG (Sigma Product No. M 6024) diluted to 1 mg/ml in Buffer III.
10. 6% PEG, M.W. approx 6,000, in Buffer III.

Assay

1. Pipette 0.1 ml of peptide/cross reactant standards to polystyrene tubes. Prepare a zero control and a blank tube, each containing 0.1 ml of Buffer I.
2. Add 0.1 ml Monoclonal Anti-Endothelin to all tubes except the blank tube; to this add 0.1 ml Buffer I.
3. Incubate at 2-8 °C for 18-24 hours (overnight).
4. Add 0.1 ml of ¹²⁵I-endothelin-1 (5-10 pg/tube) to all tubes.
5. Prepare two empty tubes for total count. Add 0.1 ml of ¹²⁵I-endothelin-1 solution to these and set aside.
6. Vortex and incubate all tubes (except total count tubes) at 2-8 °C for 18-24 hours (overnight).
7. Add 0.1 ml of EDTA solution to all tubes (except total count tubes).
8. Add 0.2 ml of diluted normal mouse serum to all tubes (except total count tubes). Mix well.

9. Add 0.2 ml of second antibody to all tubes (except total count tubes). Mix well.
10. Add 0.5 ml of polyethylene glycol solution to all tubes (except total count tubes). Mix well.
11. Centrifuge all assay tubes (except total count tubes) at 3,000 rpm at 2-8 °C for 15 minutes.
12. Carefully aspirate off the supernatants (except total count tubes).
13. Count the precipitate in a gamma counter. For each standard tube calculate the % zero bound (B/Bo) as follows:

$\% B/BO = \frac{\text{cpm in standard (or sample)} - \text{cpm in blank}}{\text{cpm in zero control} - \text{cpm in blank}} \times 100$

Generate a standard curve by plotting the % B/Bo against the log dose of standards (ng/ml).

Product Profile

Titers

1. An antibody titer of at least 1:1,000 was determined by indirect ELISA, using polyvinyl microtiter plate precoated with endothelin-1 (100 ng/ml).
2. An antibody titer of at least 1:20,000 was determined by a second antibody and polyethylene glycol RIA, using 5-10 pg/tube of ¹²⁵I-labeled endothelin-1. In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Uses

Monoclonal Anti-Endothelin may be used for the localization of endothelins (ET-1, ET-2 and ET-3), using various immunochemical assays including ELISA, dot blot, RIA and immunohistochemistry.

Sensitivity

The sensitivity of a RIA system will depend on the methodological approach as well as on the reagents. The suggested RIA sensitivity is 300 pg endothelin/tube.

RIA Affinity Constant

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

$$K_a = 2 \times 10^9 \text{ L/M}$$

Specificity

Monoclonal Anti-Endothelin reacts in radioimmunoassay (RIA) with human and porcine endothelin-1 (ET-1), human endothelin-2 (ET-2), and human and rat endothelin-3 (ET-3). Cross-reactivity is observed with sarafotoxin S6c and mouse-endothelin. No cross-reactivity is observed

with human big endothelin-38 and rat big endothelin-39, human atrial natriuretic peptide, or human angiotensin I. The product is also reactive in ELISA, competitive ELISA, dot blot and immunohistochemical staining of acetone-fixed, frozen sections.

References

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