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ProductInformation

Anti-Leukotriene B_4
Developed in Rabbit
Whole Antiserum

Product No. **L 8649**

Product Description

Antiserum to Leukotriene B_4 is developed in rabbit using leukotriene B_4-BSA as the immunogen. The product is offered as lyophilized whole antiserum.*

Leukotrienes (LT) are a group of biological mediators derived from arachidonic acid which is released from membrane phospholipids upon various cell stimulations. Leukotriene A_4 (LTA_4) an unstable epoxid is the primary derivative formed by oxygenation. It can be converted enzymatically by hydration to LTB_4 or by addition of glutathione to LTC_4. LTC_4 is metabolized to LTD_4 and LTE_4 by successive elimination of an α-glutamyl residue and glycine. The leukotrienes have potent biological actions in a number of areas and essentially participate in host defense reactions and pathophysiological conditions such as inflammation and immediate hypersensitivity. Studies also suggest a neuroendocrine role for leukotrienes. LTB_4 is leukotactic and has the leukocyte as its primary target where it causes degranulation, PMN extravasation and plasma exudation, whereas the cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4) are myotropic and primarily affect smooth muscle and other cells with contractile capacity. Thus LTB_4, which is a calcium ionophore, causes adhesion and chemotactic movement of leukocytes and stimulates aggregation, enzyme release and generation of superoxide in neutrophils. On the other hand, the cysteinyl-containing leukotrienes are potent bronchoconstrictors, increase permeability in post capillary venules and stimulate mucous secretion, following their release from lung tissue upon exposure to specific allergens. As leukotrienes have been implicated in a wide variety of diseases and pathophysiological conditions, the potential applications of a specific antibody are considerable.

Reconstitution and Dilution

Reconstitute with 5.0 ml of 0.01 M sodium phosphate buffered saline, pH 7.4, containing 0.1% BSA and 0.1% sodium azide, this is the stock antiserum solution. A 1:10 dilution of the stock antiserum (in the same buffer) yields the working dilution.

RIA Characterization

The product is characterized using a dextran coated charcoal radioimmunoassay (RIA) protocol where 0.5 ml of diluted antibody is found to bind at least 40% of 4-6 picograms of tritiated leukotriene B_4 with a specific acitivity of approximately 200 Ci/mmole.

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

Reagents

(A) Buffer: phosphate buffered saline (PBS) (10 mM phosphate, 150 mM NaCl, pH 7.4, containing 0.1% BSA and 0.1% sodium azide).
(B) Dextran-Coated Charcoal: 1.0% (w/v) activated charcoal (Product No.C 5260) and 0.1% Dextran (Product No. D 1390) in buffer without BSA. Mix for 1 hour at 4 °C prior to use.
(C) Standards: Prepare a standard solution of 1 µg/ml of LTB_4 in absolute ethanol. Dilute an aliquot in buffer to a concentration of 10 ng/ml. Six further serial doubling dilutions are prepared in buffer from the 10 ng/ml standard to give the following concentrations: 5.0, 2.5, 1.25, 0.63, 0.31, and 0.15 ng/ml.
(D) Radio-labeled leukotriene: Prepare a fresh solution of 50,000-80,000 dpm/ml of tritiated leukotriene (approximately 200 Ci/mmole) in buffer.

RIA Protocol

1. Pipet in duplicate 0.1 ml of standards to assay tubes. Prepare a zero control, a blank and a total tube.
2. Add 0.5 ml of diluted antibody to all tubes except the total and the blank, to these two tubes add 0.5 ml of buffer.
3. Incubate all tubes at 4 °C for 30 minutes.
4. Add 0.1 ml of tritiated leukotriene to all tubes and incubate at 4 °C for 60 minutes.
5. Add 0.2 ml (cold) dextran coated charcoal solution to each tube except the total tube, to this tube add 0.2 ml of cold buffer.

6. Vortex all tubes. Incubate at 4 °C for 10 minutes and then centrifuge at 4 °C for 10 minutes at 3,000 rpm.
7. Remove 0.25 ml of the supernatant and add 3 ml of scintillation fluid. Count in a liquid scintillation counter.

Calculations

1. Convert all counts to counts per minute (cpm).
2. Correct all count rates by subtracting the non-specific binding blank tube from the average of each duplicated tube.
3. Calculate the percent bound (%B) for the standards and samples as follows:
   %B = (Corrected cpm/corrected zero) x 100
4. On semilog graph paper, plot the %B for each standard against the log-dose of the standard i.e. picograms of leukotriene added.

Specificity

Specificity of the antibody is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross reactivity in the dextran coated charcoal ^{3}H RIA is as follows:

Cross Reactant	**% Cross reactivity**
LTB_4	100%
LTA_4	<0.1%
LTC_4	<0.1%
LTD_4	<0.1%
LTE_4	<0.1%
LTF_4	<0.1%
PGA_1	<0.1%
PGA_2	<0.1%
PGB_1	<0.1%
PGB_2	<0.1%
PGD_2	<0.1%
PGE_1	<0.1%
PGE_2	<0.1%
PGF_{1a}	<0.1%
PGF_{2a}	<0.1%
6-Keto-PGF_{1a}	<0.1%
13,14-Dihydro-15-Keto-PGF_{2a}	<0.1%
13,14-Dihydro-15-Keto-PGE_2	<0.1%
TXB_2	<0.1%
5-Hete	<0.1%
12-(S)-Hete	<0.1%
12-(R)-Hete	<0.1%
15-(S)-Hete	<0.1%
Arachidonic acid	<0.1%

Sensitivity

Sensitivity is defined as the 90% intercept of a B/Bo standard curve. In the ^{3}H RIA system, the sensitivity has been found to be 10 pg per tube.

Affinity Constant

The affinity constant (Ka) is determined by a Scatchard plot. Ka = 5-50 x 10^9 L/mole.

Storage

Store at 2-8 °C. After reconstitution keep stock solution at –20 °C. Avoid repeated freezing and thawing. Antiserum diluted to the working dilution should be discarded if unused within 12 hours.

* Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). Due to the PVP 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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