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ProductInformation

ANTI-PROSTAGLANDIN E_2
Developed in Rabbit
Whole Antiserum

Product No. **P 5164**

The antiserum is developed in rabbit using prostaglandin$_2$-BSA as the immunogen. The product is provided as a pre-diluted antiserum that has been lyophilized from a solution containing 0.02% sodium azide as a preservative.* This antiserum does not discriminate between prostaglandin E_1 (PG E_1) and prostaglandin E_2 (PG E_2).

Reconstitution and Dilution

1. Stock Solution: To one vial of lyophilized powder add 5.0 ml of 0.01 M sodium phosphate buffered saline, pH 7.4, containing 0.1% BSA and 0.1% sodium azide. Rotate vial gently until powder is dissolved.
2. Working Solution: To obtain the number of tests indicated on the vial further dilute the reconstituted antiserum 10-fold with the buffer used to prepare the stock solution.

Storage

Prior to reconstitution store at 2-8 °C.
After reconstitution:

1. Stock Solution: Separate into aliquots and freeze. Repeated freezing and thawing is not recommended.
2. Working Solution: Discard if unused within 12 hours.

RIA SYSTEM

RIA Characterization

The antiserum is characterized utilizing the following dextran coated charcoal radioimmunoassay (RIA) protocol, where 0.5 ml of reconstituted and diluted antiserum has been found to bind at least 40% of 5-10 picograms of tritiated (^{3}H) PG E_2 with a specific activity of approximately 160 Ci/mole. .

It is recommended that the antiserum first be evaluated in the assay system described due to differences in systems and procedures.

RIA Reagents

(A) Standards: Prepare a stock standard solution of 1 µg/ml PG E_2 (Product No. P 5640) in absolute ethanol. Dilute a portion of the stock solution with buffer (B) to a concentration of 1000 pg/0.1ml. This is further diluted in buffer (B) to obtain standard solutions at the following concentrations: 500, 250, 125, 63, 31, and 15 pg/0.1 ml.

(B) Dilution buffer: 0.01 M phosphate buffered saline, pH 7.4 containing 0.1% BSA (Product No. A 7030) and 0.1% sodium azide.

(C) Dextran coated charcoal suspension: 1.0% activated charcoal untreated powder 100-400 mesh (Product No. C 3345), 0.1% dextran approximate average molecular weight 70,000 (Product No. D 1390) in buffer (B). It is important that the dextran be in solution before the addition of charcoal. The dextran coated charcoal suspension should be stirred and kept at 0 °C in ice-water for at least 30 minutes before and during use.

RIA Protocol

1. In polypropylene test tubes add 0.1 ml sample or standard (A) and 0.5 ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 30 minutes at 4 °C.
4. Add 0.1 ml tritiated radioactive tracer diluted in dilution buffer (B).
5. Vortex the tubes.
6. Incubate for 1 hour at 4 °C.
7. Rapidly add 0.2 ml cold dextran coated charcoal suspension (C) to each tube.
8. Vortex the tubes.
9. Incubate for 10 minutes at 0 °C in ice-water.
10. Centrifuge at 2000 x g for 15 minutes at 4 °C.
11. Remove supernatant from each tube, add scintillation cocktail to the supernatant and determine the amount of radioactivity present.

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 described RIA system is as follows:

Cross-Reactant	**%Cross Reactivity**
Prostaglandin A_1 (PG A_1)	<50
PG A_2	<50
PG B_1	<50
PG B_2	<50
PG E_1	Min.100
PG E_2	Min.100
PG $F_{1\alpha}$	<20
PG $F_{2\alpha}$	<20

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 15 pg/tube.

RIA Affinity Constant
The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.
K_a = 1-10 x10^9 L/mole.

PG E_2 Levels

1. Females	28.0-35.2 pg/0.1 ml
2. Males	30.5-45.1 pg/0.1 ml

Bibliography
1. Jaffe, B.M. and H.R. Behrman, in Methods of Hormone Radioimmunoassay (Jaffe, B.M. and H.R. Behrman eds., Academic Press, New York), p19 (1974).

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

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