SIGMA CHEMICAL COMPANY

Product No. P-1791
Lot 065H4846

Anti-6-Ketoprostaglandin $F_{1\alpha}$
Developed in Rabbit
Whole Antiserum

The antiserum is developed in rabbit using 6-keto-prostaglandin-$F_{1\alpha}$-BSA as the immunogen. The product is provided as a pre-diluted antiserum that has been lyophilized with no preservative added.

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 30% of 5 - 10 picograms of tritiated (^{3}H) 6-keto-PG $F_{1\alpha}$ with a specific activity of approximately 125 Ci/mmole.

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 $F_{1\alpha}$ (Sigma Product No. K-2626) in buffer (B). Dilute a portion of the stock solution with buffer (B) to a concentration of 1000 pg/0.1 ml. 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 (Sigma Product No. A-7030) and 0.1% sodium azide.

(C) Dextran coated charcoal suspension: 1.0% activated charcoal untreated powder 100-400 mesh (Sigma Product No. C-5260), 0.1% dextran approximate average molecular weight 70,000 (Sigma 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 buffer (B).
5. Vortex the tubes.
6. Incubate for 3 hours 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)	2.5
PG A_2	1.8
PG B_1	0.7
PG B_2	<0.1
PG D_2	0.5
PG E_1	13
PG E_2	10
PG $F_{1\alpha}$	9
PG $F_{2\alpha}$	8
13-14-Dihydro-15-keto-PG E_2	<0.1
Thromboxane B_2	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 10 pg/tube.

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.
$K_a = 2.0 \times 10^{10}$ L/mole.

Uses

The product is useful for determination of 6-keto-PG $F_{1\alpha}$ which is the nonenzymatic hydrolysis product of prostacyclin (PG I_2). Prostacyclin is the main product of arachidonic acid in all arteries and veins and is chemically unstable, being hydrolysed at physiological pH and temperature, to 6-keto-PG $F_{1\alpha}$ with a considerable loss in biological activity. Prostacyclin inhibits platelet aggregation and promotes vasodilation by influencing the activity of adenylate cyclase. The measurement of 6-keto-PG $F_{1\alpha}$ is likely to be of great interest and value in attempting to explain its diverse action and metabolic activity in tissues and plasma. It has also been found in urine, thus its excretion rate may reflect renal synthesis.

References

1. Wilson, T.W., et al., J. Chromatography, **306**, 351 (1984).
2. Adaikan, P.G., et al., Prostaglandins, **27**, 505 (1984).
3. Mitchell, J.R.A., Br. Med. J., **287**, 1824 (1983).

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