

Product No. E-2885
Lot 116H4881

Anti-17 β -Estradiol
Developed in Rabbit
Whole Antiserum

This antiserum is developed in rabbit using 17 β -estradiol-6-BSA as the immunogen. The product is diluted antiserum that has been lyophilized.*

Reconstitution Instructions

To one vial of lyophilized powder, add 5.0 ml Tris-HCl buffer (0.05 M, pH 8.0) containing 0.1 M NaCl, 0.1% gelatin and 0.1% sodium azide. Rotate gently until the powder dissolves. This is the stock antiserum solution. To obtain the number of tests indicated on the vial, the reconstituted antiserum should be further diluted 10-fold with the same buffer to produce the working dilution of the antiserum.

Storage

Prior to reconstitution, store at 2-8°C. After reconstitution, the stock solution should be separated into aliquots and frozen. The working dilution of the antiserum should be discarded if unused within 12 hours. Repeated freezing and thawing is not recommended.

Tests Per Vial

The number of tests per vial is determined 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 17 β -estradiol with a specific activity of approximately 100 ci/mmoles.

The number of tests per vial and subsequent lot specific data indicate the performance of the antiserum in the assay system utilized at Sigma. It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in assay systems and procedures.

Reagents

- (A) Standard: Prepare a stock standard solution of 1 μ g/ml 17 β -estradiol (Sigma Product No. E-8875) in absolute ethanol. Dilute a portion of the stock standard with buffer (B) to a concentration of 500 pg/0.1 ml. The 500 pg/0.1 ml standard should be further diluted in buffer (B) to obtain standard solutions at the following concentrations: 7.5, 15, 31, 63, 125, 250, and 500 pg/0.1 ml.
- (B) Tris-HCL (Sigma Product No. T-3253) buffer (0.05 M, pH 8.0) containing 0.1 M NaCl, 0.1% Gelatin (Sigma Product No. G-2500) and 0.1% sodium azide.
- (C) Dextran coated charcoal suspension: Buffer (B) containing 0.5% activated charcoal untreated powder 100-400 mesh (Sigma Product No. C-5260) and 0.05% dextran approximate average molecular weight 70,000 (Sigma Product No. D-1390). It is important that the dextran be in solution before addition of the charcoal and that the dextran coated charcoal suspension 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 antiserum reconstituted and diluted in buffer (B) to the working dilution.
2. Vortex the tubes.
3. Incubate for 30 minutes at room temperature.
4. Add 0.1 ml tracer prepared fresh in buffer (B).
5. Vortex the tubes.
6. Incubate for 60 minutes at 37°C.
7. Cool the tubes for 15 minutes at 4°C.
8. Rapidly add 0.2 ml cold dextran coated charcoal suspension (C) to each tube.
9. Vortex the tubes.
10. Incubate for 10 minutes at 0°C in ice/water.
11. Centrifuge at 2000 x g for 15 minutes at 4°C.
12. Remove supernatant from each tube, add scintillation cocktail to the supernatant, and determine the amount of radioactivity present.

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 Sigma's dextran coated charcoal ³H-RIA system is as follows:

Cross-Reactant	% Cross-Reactivity
Equilenin	1.0
Equilin	<0.01
17 α -Estradiol	5.0
Estriol	1.3
Estrone	4.8
17 α -Ethinylestradiol	0.3
6 α -Hydroxyestradiol	82
6-Ketoestradiol	141
Progesterone	<0.1
Testosterone	<0.1

Sensitivity

Sensitivity is defined as the 90% intercept of a B/B₀ standard curve. Using Sigma's RIA system the sensitivity has been found to be 5 pg per tube.

Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using Sigma's RIA system.

$$K_a = 7.3 \times 10^9 \text{ L/mole.}$$

17 β -Estradiol Levels^{2,3}

	pg/0.1 ml
1. Men	1.7-4.1
2. Women	
(A) Follicular phase	1.8-21.4
(B) Pre-ovulatory phase	4.7-24.1
(C) Post-ovulatory phase	2.6-14.8
(D) Pregnant	
i) 12 weeks	288
ii) 24 weeks	252
iii) 37 weeks	3,500

References

1. Lindner, H., et al., *Steroids*, **19**, 357 (1972).
2. Emmet, Y., et al., *Acta Endocrin.*, **69**, 567 (1972).
3. Wu, C., and L. Lundy, *Steroids*, **18**, 91 (1971).

* 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.