

Product No. D-8886
Lot 076H6655

Anti-Dehydroepiandrosterone (DHEA)

Developed in Rabbit
Whole Antiserum

Antiserum was developed in rabbit using DHEA-15 α CH₂-CO-BSA as the immunogen. The product is provided lyophilized.

Reconstitution

To obtain number of tests indicated on vial reconstitute with 11 ml of 0.1 M phosphate buffered saline, pH 7.4, containing 0.1 M gelatin and 0.01% sodium azide.

This antiserum is not assayed by Sigma. The supplier has furnished Sigma with the following information.

Cross Reactivity (B/B₀ = 50%)

| | <u>% Cross Reactivity</u> |
|--|---------------------------|
| Testosterone | <0.01 |
| Dihydrotestosterone | 0.05 |
| 5 α -Androstane-diol-3 β | <0.01 |
| 5 α -Androstane-diol-3 α | <0.01 |
| Androstenediol | 0.08 |
| Androsterone | <0.01 |
| Epiandrosterone | <0.01 |
| 5 β -Androstane-3 α | <0.01 |
| Estradiol | <0.01 |
| Progesterone | <0.01 |
| Pregnenolone | 0.1 |
| Sulfate DHEA | 0.02 |
| Cholesterol | <0.01 |
| Estrone | <0.01 |

Binding Parameters

B₀ = 41% of 23 pg of tritiated (³H) tracer
 IC₈₀ = 1 pg/tube
 IC₅₀ = 6.5 pg/tube
 IC₂₀ = 40 pg/tube

Affinity Constant: K_a = 1.1 x 10¹⁰ L/Mole

Reagents

- 0.1 M Phosphate buffered saline, pH 7.4, containing 1% gelatin and 0.01% sodium azide.
- Dextran coated charcoal suspension: 5 grams of charcoal and 0.5 grams dextran in 1 liter of buffer A. It is important that the dextran be in solution before the addition of charcoal. The suspension should be stirred and kept at 0°C in an ice-water bath for at least 30 minutes before and during use.

RIA Protocol

- In polypropylene test tubes add 0.1ml sample or standard, 0.1ml tritiated tracer and 0.1ml diluted antiserum.
- Vortex the tubes.
- Allow to equilibrate at room temperature for 30 minutes, then at least 15 minutes at 0°C in an ice/water bath.
- Add 1 ml cold dextran charcoal solution, keep in ice-water bath for 10 minutes.
- Centrifuge at 2000 x g for 10 minutes at 4°C.
- Remove supernatant from each tube. Add scintillation fluid to supernatant and determine the amount of radioactivity present in the supernatant.

Storage

For continuous use, store at 2-8°C. For extended storage, the solution may be frozen 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.

Bibliography

Kouznetsva, B., and F. Dray, J. Steroid Biochem., **9**, 349 (1978).