

Product No. D7782
Anti-Digoxin
Lot 077H4826

Developed in Rabbit
Whole Antiserum

The antiserum is developed in rabbit using digoxin-BSA as the immunogen. The product is provided as a undiluted antiserum containing 0.1% sodium azide (see MSDS)* as a preservative.

Working Dilution

Dilute the antiserum to a working dilution of 1:12,500 in 0.01M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide.

Storage

Store the undiluted antiserum at -20°C, in working aliquots. Repeated freezing and thawing is **not** recommended.

RIA SYSTEM

RIA Characterization

The antiserum is characterized utilizing the following dextran coated charcoal radioimmunoassay (RIA) protocol, where 0.5 ml of diluted antiserum has been found to bind at least 40% of 70 picograms of tritiated (³H) digoxin with a specific activity of approximately 10-20 Ci/mmol.

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 1µg/ml digoxin (Sigma Product No. D6003) in absolute ethanol. Dilute a portion of the stock solution with normal human serum to a concentration of 250 pg/0.1ml. This is further diluted in normal human serum to obtain standard solutions at the following concentrations: 125, 63, 31 and 15 pg/0.1ml.
- (B) Phosphate Buffered Saline, 0.01M, pH 7.4, containing 0.1% sodium azide.

- (C) Dextran coated charcoal suspension: 1.0% activated charcoal untreated powder 100-400 mesh (Sigma Product No. C5260), 0.1% dextran approximate average molecular weight 70,000 (Sigma Product No. D1390) 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 0.1ml sample or standard (A) and 0.5ml diluted antiserum.
2. Vortex the tubes.
3. Incubate for 30 minutes at room temperature.
4. Add 0.1ml tritiated radioactive tracer diluted in dilution buffer (B).
5. Vortex the tubes.
6. Incubate for 1 hour at room temperature.
7. Cool the tubes for 15 minutes at 4°C.
8. Rapidly add 0.2ml 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.

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B₀ standard curve. In the above system the sensitivity has been found to be 15 pg digoxin per tube..

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**

Aldospirone	0.1
Cortisol	<0.1
Digitoxin	0.74
17 β -Estradiol	<0.1
Progesterone	<0.1
Testosterone	<0.1

*Due to the 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.

RIA Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.

$K_a = 2.7 \times 10^{10}$ L/mole.