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## Product Information

### ANTI-DIGITOXIN Whole Antiserum

Product No. **D7657**

#### Product Description

The anti-digitoxin is developed in rabbit using digitoxin-BSA as the immunogen.

#### Reagents

The product is provided as a undiluted antiserum containing 0.1% sodium azide as a preservative.

#### Precautions and Disclaimer

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.

#### Working Dilution

Dilute the antiserum to a working dilution of 1:5,000 in 0.01M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide.

#### Storage/Stability

Store the undiluted antiserum at  $-20^{\circ}\text{C}$ , 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 30% of 100 picograms of tritiated ( $^3\text{H}$ ) digitoxin with a specific activity of approximately 15 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  $1\mu\text{g/ml}$  digitoxin (Product No. D 5878) in absolute ethanol. Dilute a portion of the stock solution with normal human serum to a concentration of 1000  $\text{pg}/0.1\text{ml}$ . This is further diluted in normal human serum to obtain standard solutions at the following concentrations: 500, 250, 125, 63, and 31  $\text{pg}/0.1\text{ml}$ .
- (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, 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 0.1ml 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/B<sub>0</sub> standard curve. In the above system the sensitivity has been found to be 15 pg/tube.

#### RI 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
Digitoxin	100
Cortisol	< 0.5
Deslanoside	<10
Digoxin	<20
Estradiol	< 0.5
Progesterone	< 0.5
Testosterone	< 0.5
Furosemide (Lasix)	< 0.5
Sodium chlorothiazide (Diuril)	< 0.1
Spirolactone	<0.1

#### A Affinity Constant

The affinity constant (K<sub>a</sub>) is determined by a Scatchard plot using the described RIA system.

$$K_a = 1-10 \times 10^9 \text{L/mole.}$$

#### Digitoxin Serum Levels

ng/ml (mean ± standard deviation)

1. Non-toxic concentration      20 ± 11 (see ref 2)  
17 ± 8 (see ref 2)
2. Toxic concentration          34 ± 18 (see ref 2)  
34 ± 6 (see ref 3)

#### References

1. Butler, V. P., Jr., and Chen, J. P., Proc. Natl. Acad. Sci., **57**, 71 (1967).
2. Beller, G. A., et al., N. Engl. J. Med., **284**, 989 (1971).
3. Smith, T. W., J. Pharmacol. Exp. Therap., **175**, 352, (1970).
4. Besch, H. R., Jr., and Watanabe, A. M., Clin. Chem., **21**, 1815 (1975).

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