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## Product Information

### Anti-L-Thyroxine (T<sub>4</sub>)

produced in rabbit, whole antiserum

Catalog Number **T2652**

### Product Description

Anti-L-Thyroxine (T<sub>4</sub>) is produced in rabbit using as immunogen L-Thyroxine-BSA (T<sub>4</sub>-BSA).

### Reagent

Supplied as whole antiserum containing 15 mM sodium azide as a preservative.

### Precautions

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage

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

### Product Profile

Radioimmunoassay (RIA): a working dilution of 1:500 is recommended.

### RIA System

### RIA Characterization

The antiserum is characterized utilizing the following secondary antibody-polyethylene glycol (PEG) RIA protocol, where 0.1ml of antiserum at the working dilution has been found to bind at least 40% of 15 picograms of iodinated T<sub>4</sub> with a specific activity of approximately 1000 μCi/μg.

It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in systems and procedures.

### RIA Reagents

- A. Standards: Prepare and freeze aliquots of a stock standard solution of 1.0 mg/ml T<sub>4</sub> free acid, Catalog Number T2376, in 0.05 M NaOH. Dilute an aliquot in 0.05 M NaOH to 25 μg/ml, this is then further diluted in T<sub>4</sub> free serum (B) to the following concentrations: 12,500, 6250, 3125, 1562, 781, 390, 195 and 98 pg/0.1ml.
- B. T<sub>4</sub> free serum: To 50 ml of normal human serum add approximately 0.7 μL of <sup>125</sup>I-T<sub>4</sub> (iodinated L-Thyroxine) with a specific activity of approximately 1200 μCi/μg so that the solution is about 300 cpm/0.1 ml. Add 10 g activated charcoal untreated powder and stir gently overnight at 4 °C. Centrifuge at 24,000 x g for 30 minutes at 4 °C. Transfer the supernatant and centrifuge an additional hour at 24,000 x g at 4 °C. Filter the supernatant through a 0.22 μm filter. There should be no more than 5% of the initial <sup>125</sup>I-T<sub>4</sub> counts remaining.
- C. 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.
- D. Dilute the antiserum in buffer (C)
- E. T<sub>4</sub> diluent: 0.075 M sodium barbital, pH 8.6, in distilled water, containing 0.05% 8-anilo-1-naphthalenesulfonic acid ammonium salt, Catalog Number A3125, 2.0% normal rabbit serum, Catalog Number R9133, and 15 mM sodium azide. Adjust the pH with concentrated sulfuric acid.
- F. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt, Catalog Number ED2SS, 0.1 M, pH 7.8 in distilled water. Adjust the pH with 10 N NaOH.
- G. Secondary antibody: Anti-Rabbit IgG, Catalog Number R0881, reconstituted in buffer (C). Dilute reconstituted antiserum 1:5 in buffer (C) for use.
- H. EDTA-secondary antibody mixture reagent: Mix equal volumes of EDTA solution (F) with diluted secondary antibody (G).
- I. PEG solution: 6% PEG, Catalog Number P2139, approximate molecular weight 8,000, in buffer (C).

### RIA Protocol

1. In polypropylene test tubes add 0.1 ml sample or standard and 0.1 ml diluted antiserum and 0.2 ml <sup>125</sup>I radioactive tracer prepared fresh in T<sub>4</sub>-diluent (E).
2. Vortex the tubes.
3. Incubate for 1 hour at 37 °C.
4. Add 0.2 ml EDTA-secondary antibody reaction mixture (H).
5. Add 0.5 ml PEG solution (I).
6. Vortex the tubes.
7. Centrifuge at 2000 x g for 15 minutes at 4 °C.
8. Remove supernatant from each tube and determine the amount of radioactivity present in the precipitate.

### 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 second antibody-PEG I<sup>125</sup> RIA system is as follows:

Cross-Reactant	%Cross-Reactivity
Diiodo-L-tyrosine	<0.01
Moniodo-L-tyrosine	<0.01
D-Thyroxine	100
3,3',5-Triiodo-L-thyronine (T <sub>3</sub> )	< 5

### 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 100 pg per tube.

### RIA Affinity Constant

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

$$K_a = 1 \times 10^8 - 1 \times 10^9 \text{ L/mole.}$$

MG,KAA,PHC 09/07-1