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Product Information

Anti-L-Thyroxine (T₄)

produced in rabbit, whole antiserum

Catalog Number **T2652**

Product Description

Anti-L-Thyroxine (T₄) is produced in rabbit using as immunogen L-Thyroxine-BSA (T₄-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 T₄ 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 T₄ 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 T₄ free serum (B) to the following concentrations: 12,500, 6250, 3125, 1562, 781, 390, 195 and 98 pg/0.1ml.
- B. T₄ free serum: To 50 ml of normal human serum add approximately 0.7 μL of ¹²⁵I-T₄ (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 ¹²⁵I-T₄ 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. T₄ 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 ¹²⁵I radioactive tracer prepared fresh in T₄-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 I¹²⁵ 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 ₃)	< 5

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 100 pg per tube.

RIA Affinity Constant

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

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

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