

ProductInformation

ANTI-TRIIODOTHYRONINE (T₃)

Developed in Rabbit

Whole Antiserum

Product No. **T2777**

Product Description

The antiserum is developed in rabbit using L-triiodo-thyronine-BSA (T₃-BSA) as the immunogen.

Reagents

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

Precautions

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.

Product Profile

Dilute the antiserum to a working dilution of 1:6000 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 second antibody-polyethylene glycol (PEG) RIA protocol, where 0.1ml of antiserum at the working dilution has been found to bind at least 40% of 10 picograms of iodinated T₃ with a specific activity of approximately 1300 $\mu\text{Ci}/\mu\text{g}$.

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 1.0mg/ml T₃ free acid (Product No. T2877) in 0.05M NaOH. Dilute an aliquot in 0.05M NaOH to 25 $\mu\text{g}/\text{ml}$, this is then further diluted in T₃ free serum (B) 250ng/ml which is then further diluted to the following concentrations: 800, 400, 200, 100, 50, 25 and 12.5 pg/0.1ml.
- B. T₃ free serum: To 50 ml of normal human serum add approximately 0.7 μl of ¹²⁵I-T₃ (iodinated with a specific activity of approximately 1200 $\mu\text{Ci}/\mu\text{g}$ so that the solution is about 300 cpm/0.1ml. Add 10 g activated charcoal untreated powder (Product No. C5260, 250-350 mesh) 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-T₃ counts remaining.
- C. 0.01M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide.
- D. T₃ diluent: 0.075M sodium barbital, pH 8.6, in distilled water, containing 0.05% 8-anilo-1-naphthalenesulfonic acid ammonium salt (Product No. A3125), 2.0% normal rabbit serum (Product No. R9133) and 0.1% sodium azide. Adjust the pH with concentrated sulfuric acid.
- E. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Product Code ED2SS), 0.1M, pH 7.8 in distilled water. Adjust the pH with 10 N NaOH.
- F. Second antibody: Goat anti-Rabbit IgG (Product No. R0881), reconstituted in buffer (C). Dilute reconstituted antiserum 1:5 in buffer (C) for use.
- G. EDTA-second antibody mixture reagent: Mix equal volumes of EDTA solution (E) with diluted second antibody (F).
- H. PEG solution: 6% PEG (Product No. P2139, approximate molecular weight 8,000) in buffer (C).

RIA Protocol

1. In polypropylene test tubes add 0.1ml sample or standard and 0.1ml diluted antiserum and 0.2ml ^{125}I radioactive tracer prepared fresh in T_3 -diluent (D).
2. Vortex the tubes.
3. Incubate for 1 hour at 37°C .
4. Add 0.2ml EDTA-second antibody reaction mixture (G).
5. Add 0.5ml PEG solution (H).
6. Vortex the tubes.
7. Centrifuge at $2000 \times g$ 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 ^{125}I RIA system is as follows:

Cross-Reactant	%Cross-Reactivity
Diiodo-L-thyronine	0.5
Diiodo-L-tyrosine	< 0.001
Monoiodo-L-tyrosine	<0.001
L-Thyroxine (T_4)	0.14
Triiodothyroacetic acid	24.0

RIA Sensitivity

Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the above system the sensitivity has been found to be 10 pg/tube.

RIA Affinity Constant

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

$$K_a = 4.5 \times 10^9 \text{ L/mole.}$$

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