

## Product Information

### MOUSE MONOCLONAL ANTI-TRIIODOTHYRONINE (T<sub>3</sub>)

Immunoglobulin Fraction of Cell Culture Supernatant  
Clone T3-10

Product Number **T3776**

#### Product Description

Monoclonal Anti-Triiodothyronine (T<sub>3</sub>) is derived from the hybridoma (T3-10) produced by the fusion of mouse myeloma cells and BALB/c mice splenocytes. Mice were immunized with 3,3',5-triiodo-L-thyronine-bovine serum albumin (T<sub>3</sub>-BSA). The antibody is of the mouse IgG1 isotype and is supplied as the immunoglobulin fraction of a cell culture supernatant.

#### Reagents

Monoclonal Anti-T<sub>3</sub> is provided as a liquid and contains 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.

#### Storage/Stability

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

#### Procedure

The antiserum is characterized utilizing the following second antibody-polyethylene glycol (PEG) RIA protocol.

#### RIA Reagents

A. Standards: Prepare and freeze aliquots of a stock standard solution of 1.0 mg/ml T<sub>3</sub> free acid (Product No. T2877) 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>3</sub> free serum (B) 25 mg/ml which is then further diluted to the following concentrations: 10,000, 5000, 2500, 1250, 625 312, 156, and 78 pg/0.1ml.

- B. T<sub>3</sub> free serum: To 50 ml of normal human serum add approximately 0.7 µl of <sup>125</sup>I-T<sub>3</sub> (iodinated with a specific activity of approximately 1200 µCi/µg 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 <sup>125</sup>I-T<sub>3</sub> counts remaining.
- C. 0.01 M phosphate buffered saline, pH 7.4, containing 0.1% sodium azide.
- D. T<sub>3</sub> diluent: 0.075 M sodium barbital, pH 8.6, in distilled water, containing 0.05% 8-anilo-1-naphthalenesulfonic acid ammonium salt (Product No. A3125), 2.0% normal mouse serum (Product No. M5905) and 0.1% sodium azide. Adjust the pH with concentrated sulfuric acid.
- E. EDTA solution: Ethylenediaminetetraacetic acid (EDTA) disodium salt (Product Code ED2SS), 0.1 M, pH 7.8, in distilled water. Adjust the pH with 10 N NaOH.
- F. Second antibody: Rabbit Anti-Mouse IgG (Product No. M6024), reconstituted to 2 mg/ml in buffer (C).
- 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 0.1 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>3</sub>-diluent (D).

2. Vortex the tubes.
3. Incubate for 1 hour at 37 °C.
4. Add 0.2 ml EDTA-second antibody reaction mixture (G).
5. Add 0.5 ml PEG solution (H).
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.

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

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