AgAbBtn + EnzAgAbBtn

Sample and fixed amount of T3 analog for a fixed number of results between the native antigen and the enzyme-antigen substrate that produces color.

Patient Specimen) is added to the streptavidin coated microwells. Upon mixing biotinylated antibody, enzyme-antigen conjugate and substrate, the antibody-bound mixture forms a sandwich complex bound to the surface.

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwells occurs. This effect separates the antibody bound fraction from the microwell.

6. Test tubes for dilution of enzyme conjugate and substrate A.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate covers for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV in VDRL test or rapid test. These screening test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory practices for handling blood products can be found in the Center for Disease Control’s “Guidelines for Microbial and Biologic Laboratories,” 2nd Edition, 1986. HHS Publication No. (CDC) 88-3839.

Safe disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a red top vacutainer tube or with lithium heparin (LH). EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assessed within this time, the sample(s) may be stored at temperatures of -20°C for up to 15-30 days. Avoid repeated thawing and freezing. When assayed in duplicate, 0.100 ml (100µl) of the specimen is required for T3.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. Each unknown, calibrator, control or specimen into the assigned wells. The results should be read at 28°C.

8.0 REAGENT PREPARATION

Note: To determine the concentration of Total T3 for an unknown, plot the readout value by 2 to obtain the thyroxine concentration.

9.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of T3 in known specimens.

1. Record the absorbance obtained from the printout of the microplate reader as illustrated in Example 1.

2. Plot the absorbance values for each dilution on a log-log graph paper. Note the corresponding Total T3 in mg/ml (or log transformed unit). The X-axis of the graph represents the log-transformed concentration and the Y-axis represents the absorbance value.

3. Connect the points with a best-fit curve (Figures 1-3).

4. To determine the concentration of Total T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the corresponding Total T3 in mg/ml (or log transformed unit).

Note: Do not use the working substrate if it looks blue. Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20-27°C).

1. Format the microplates’ wells for each serum calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.050ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned wells.

3. Add 0.500ml (500µl) of Working Enzyme Reagent solution to the appropriate wells (see Reagent Preparation Section).

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Incubate at room temperature.

6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent filter paper.

7. Add 0.300ml (300µl) of wash buffer (see Reagent Preparation Section, decant (tap and blow) or aspirate. Repeat two (2) additional times for a total of three (3) washes. An automatic or manual plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoid air bubbles) to dispense the wash buffer. Do not allow the wash buffer to overflow. Additional washes are recommended.

8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order and in the same volumes to avoid reaction time differences between wells.

Note: DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.

11. Incubate at room temperature for fifteen (15) minutes.

12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 2 minutes.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 680nm). The readings should be within thirty (30) minutes of adding the stop solution.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact protocol, is essential. Any deviation from Monobind’s IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation
1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other diagnostic information.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies could be one of these interference types. It is highly recommended to have these interference types be further identified. For problems for all kinds of immunoassays (Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays’ Clin Chem 1988;34:247-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Total serum T3 concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG. Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.
8. Total serum T3 values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3U uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevation in T3 is caused by TBG variation.
9. A decrease in i3T values is found with protein-wasting diseases, certain cancer diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions which affect i3T values has been compiled by the Journal of the American Association of Clinical Chemists.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES
A study of eutrophic adult population was undertaken to determine expected values. The mean (R) values, standard deviations (d) and expected ranges (±2d) are presented in Table 1.

14.0 PERFORMANCE CHARACTERISTICS
14.1 Precision
The within and between assay precision of the Total T3 SBS AccuBind® ELISA Test System was determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

14.2 Sensitivity
The Total T3 SBS AccuBind® ELISA Test System has a sensitivity of 0.04 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml standard calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy
The Total T3 SBS AccuBind® ELISA Test System was compared with a least square regression equation and the correlation coefficient were computed for the ELISA in comparison with the reference methods. The data obtained are displayed in Table 4.

15.0 REFERENCES