Monobind Inc.
Lake Forest, CA 92630, USA

VAST™
Versatile Analyte System Technology
3 Analytes 1 Kit
ELISA Microwells

# Free Thyroxine, Free Triiodothyronine, Thyrotropin (Free T3/Free T4/TSH VAST®) Free Thyroid Panel

***Product Code: 7025-300***

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Free Thyroxine; Free Triiodothyronine; Thyrotropin Concentration for a comprehensive thyroid status of a Human Serum or Plasma sample by a Microplate Enzyme Immunoassay, Colorimetric**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurements of thyroid hormones (fT3, fT4 and TSH) are generally regarded as invaluable *in-vitro* diagnostic tests for assessing thyroid function.

The Combination Free Thyroid Panel provides the convenience of combination calibrators, universal plate and flexible reagent selection allowing technicians to perform a variety of assay designs. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-fT4 (fT3) conjugate and biotinylated fT4 or fT3 antibody are added, and the reactants are mixed. In the case of TSH, the biotinylated and enzyme conjugate are added in one step. A reaction results between the enzyme conjugate, biotinylated conjugate and the native thyroid hormone (fT3, fT4 or TSH) for the antibody combining sites. Immobilization takes place through the reaction of the incorporated biotin and streptavidin coated on the well. After the completion of the required incubation period, the bound enzyme conjugate is separated from the unbound enzyme conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known thyroid hormone concentration(s) permits construction of a graph of activity and concentration. From comparison to the dose response curve(s), an unknown specimen's activity can be correlated with hormone concentration.

## 3.0 PRINCIPLE

**Competitive Enzyme Immunoassay (fT3 and fT4) – Type 7**
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate, native antigen and a substrate that produces color.

Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ag = Native Antigen (Variable Quantity)
$^{Enz}Ag$ = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody-bound fraction, measured by reaction with TMB, is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

**Immunoenzymometric assay (TSH) - TYPE 3**
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{TSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{TSH}$ = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ =Enzyme -Polyclonal Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$= Antigen-Antibodies Sandwich Complex
$k_a$ = Rate Constant of Association
$k_{-a}$ = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction, measured by reaction with tetramethylbenzidine (TMB), is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 REAGENTS

**Reagents for 2 X 96 well Microplate, provided**

**A. Combi-Cal® Free Thyroid Calibrators - 1ml/vial- Icon A-F**
Six (6) vials of Thyroid Combi-Cal™ human serum calibrators dispensed in vials with the concentrations as listed in the Table. Store at 2-8°C. A preservative has been added
***Exact levels are given on the labels on a lot specific basis***

| Analyte | fT3 (pg/ml) | fT4 (ng/dl) | TSH (µlU/ml) |
|---|---|---|---|
| A | 0 | 0 | 0 |
| B | 1.3 | 0.5 | 0.5 |
| C | 3.0 | 1.2 | 2.5 |
| D | 8.0 | 2.4 | 10.0 |
| E | 12.0 | 4.2 | 20.0 |
| F | 22.0 | 7.6 | 40.0 |

**B. Strept fT4 Enzyme Reagent – 7 ml/vial - Icon** (E)
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

**C. Strept fT3 Enzyme Reagent – 7 ml/vial - Icon** (E)
One (1) vial of triiodothyronine -horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C

**D. TSH Enzyme Reagent — 13ml/vial - Icon** (E)
One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

**E. Strept fT4 Biotin Reagent – 7ml/vial – Icon ∇**
One (1) vial of biotinylated anti-thyroxine (sheep) reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

**F. Strept fT3 Biotin Reagent – 7ml/vial – Icon ∇**
One (1) vial of biotinylated anti-triiothyronine (sheep) reagent in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

**G. Streptavidin Coated Microplate – 2 x 96 wells – Icon ⇓**
Two (2) 96-well microplates coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**H. Wash Solution Concentrate – 20ml - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

**I. Substrate Reagent – 2 X 12ml/vial - Icon $S^N$**
Two (2) amber bottles contain tetramethylbenzidine (TMB) and hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C.

**J. Stop Solution – 2 x 8ml/vial - Icon** (STOP)
Two (2) vial contains a strong acid (0.5M $H_2SO_4$). Store at 2-8°C.

**K. Product Insert.**

**Note 1:** TSH concentrations were calibrated using a reference preparation, which was assayed against the WHO 2nd. IRP 80/558

**Note 2**: Do not use reagents beyond the kit expiration date.

**Note 3:** Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**

**Note 4:** Above reagents are for a 192 well microplate kit. For other kit configurations, refer to the chart at the end of the instructions.

### 4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate dilutions.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for dilution of samples if required.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover 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 Antibodies by FDA required tests. Since no known 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 procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

**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 plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin.. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

**In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.**

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.05ml (50µl) of the specimen is required for fT4 and TSH analysis and 0.10ml (100µl) is required for fT3 analysis.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8.0 REAGENT PREPARATION

**1. Wash Buffer**
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.

***Note: Do not use reagents that are contaminated or have bacteria growth***

## 9.0 TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).*
***Test Procedure should be performed by a skilled individual or trained professional****

1. Format the microplates' wells for each serum calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well for fT4**. Pipette 0.050ml (50µl) for fT3. Pipette 0.025ml (25µl) for TSH.**
3. Add 0.050 ml (50µl) of Enzyme Reagent fT4 **or fT3** to the appropriate wells. **For TSH, add 0.100ml (100µl) of TSH Enzyme Reagent and skip steps 4 and 5.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Add 0.050 ml (50µl) of biotinylated x-fT4 **or (x-fT3)** reagent to the appropriate wells.
6. Swirl the microplate gently for 20-30 seconds to mix and cover.
7. Incubate 60 minutes at room temperature.

8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) 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 (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

**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 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

**Note**: For reassaying specimens with concentrations greater than highest calibrator, dilute 0.0125ml (12.5µl for fT4 and TSH) or 0.025ml (25µl for fT3) of the specimen and 0.0125ml (12.5µl for fT4and TSH) or 0.025ml (25µl for fT3) of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the thyroxine concentration.

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyroid hormones in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1 – fT4, Example 2 - fT3 or Example 3 - TSH.
2. Plot the absorbance for each duplicate serum reference versus the corresponding fT4 in ng/dl , (fT3 concentration in pg/ml - TSH in µIU/ml) on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve (Figures 1-3).
4. To determine the concentration of fT4, fT3, or TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration in ng/dl (fT4), pg/ml (fT3), and µIU/ml (TSH) from the horizontal axis of the graph. The duplicates of the unknown may be averaged as indicated. In the following example for fT4, the average absorbance 0.792 intersects the calibrator curve at 1.86 ng/dl fT4 concentration (See Figure 1).

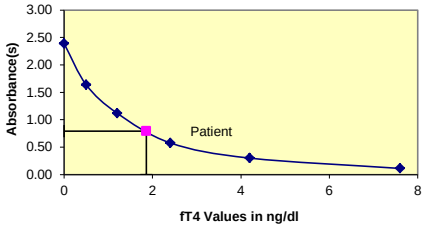
**Note:** Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

The data presented in Example 1-3 and Figure 1-3 are for illustration only and **should not** be used in lieu of calibration curve prepared with each assay.

**EXAMPLE 1 – fT4**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (ng/dl) |
|---|---|---|---|---|
| Cal A | A1 | 2.338 | 2.389 | 0.0 |
| | A2 | 2.441 | | |
| Cal B | B1 | 1.626 | 1.638 | 0.5 |
| | B2 | 1.651 | | |
| Cal C | C1 | 1.111 | 1.119 | 1.2 |
| | C2 | 1.26 | | |
| Cal D | D1 | 0.590 | 0.577 | 2.4 |
| | D2 | 0.563 | | |
| Cal E | E1 | 0.308 | 0.299 | 4.2 |
| | E2 | 0.291 | | |
| Cal F | F1 | 0.113 | 0.111 | 7.6 |
| | F2 | 0.110 | | |
| Patient | H1 | 0.818 | 0.792 | 1.86 |
| | H2 | 0.765 | | |

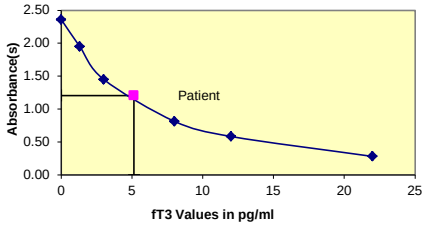
**Figure 1**




**EXAMPLE 2 – fT3**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (pg/ml) |
|---|---|---|---|---|
| Cal A | A1 | 2.365 | 2.358 | 0 |
| | A2 | 2.351 | | |
| Cal B | B1 | 1.960 | 1.950 | 1.3 |
| | B2 | 1.940 | | |
| Cal C | C1 | 1.457 | 1.449 | 3.0 |
| | C2 | 1.442 | | |
| Cal D | D1 | 0.829 | 0.812 | 8.0 |
| | D2 | 0.795 | | |
| Cal E | E1 | 0.592 | 0.582 | 12.0 |
| | E2 | 0.571 | | |
| Cal F | F1 | 0.281 | 0.279 | 22.0 |
| | F2 | 0.278 | | |
| Patient | H1 | 1.211 | 1.206 | 5.14 |
| | H2 | 1.200 | | |

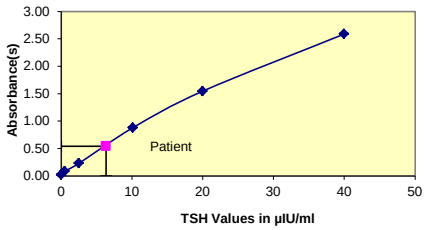
**Figure 1**




**EXAMPLE 3 - TSH**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (µIU/ml) |
|---|---|---|---|---|
| Cal A | A1 | 0.024 | 0.023 | 0 |
| | A2 | 0.023 | | |
| Cal B | B1 | 0.081 | 0.082 | 0.5 |
| | B2 | 0.084 | | |
| Cal C | C1 | 0.229 | 0.230 | 2.5 |
| | C2 | 0.231 | | |
| Cal D | D1 | 0.922 | 0.877 | 10.1 |
| | D2 | 0.832 | | |
| Cal E | E1 | 1.594 | 1.546 | 20.0 |
| | E2 | 1.498 | | |
| Cal F | F1 | 2.661 | 2.588 | 40.0 |
| | F2 | 2.516 | | |
| Patient | H1 | 0.560 | 0.538 | 6.34 |
| | H2 | 0.516 | | |

**Figure 1**




## 11.0 Q.C. PARAMETERS

**In order for the assay results to be considered valid the following criteria should be met:**

1. The absorbance (OD) of calibrator A for fT3 and fT4 of calibrator F for TSH should be $\geq$ 1.3
2. Four out of six quality control pools should be within the established ranges.

## 12.0 RISK ANALYSIS

*The MSDS and Risk Analysis for this product are available upon request from Monobind Inc.*

### 12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with concentrations greater than the highest calibrator can be diluted; dilute 12.5µl (fT4 and TSH) or 25µl (fT3) of the specimen and 12.5µl (fT4 and TSH) or 25µl (fT3) of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the concentration.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. 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.
12. 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.
13. 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 determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). 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 thyroxine 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 (3, 4). Thus, total thyroxine concentration alone is not sufficient to assess clinical status.
8. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated T4 is caused by TBG variation.A decrease in total thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total thyroxine 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 euthyroid adult population was undertaken to determine expected values. The mean (R) values, standard deviations (**σ**) and expected ranges (±2**σ**) are presented in Table 1 for fT4 and Table 2 for fT3. A nonparametric method (95% Percentile Estimate) was used for TSH in Table 3.

**TABLE I - Expected Values – (fT4) (in ng/dl)**

| | Adult | Pregnancy |
|---|---|---|
| Mean (X) | 1.40 | 1.50 |
| Std. Dev (σ) | 0.3 | 0.37 |
| Expected Ranges (±2σ) | 0.8-2.0 | 0.76-2.24 |

**TABLE 2 - Expected Values – (fT3) (in pg/ml)**

| | Adult | Pregnancy |
|---|---|---|
| Mean (X) | 2.80 | 3.0 |
| Std. Dev (σ) | 0.375 | 0.6 |
| Expected Ranges (±2σ) | 1.4-4.2 | 1.8-4.2 |

**TABLE 3 - Expected Values – (TSH) (in µIU/ml)**

| | |
|---|---|
| Low Normal Range | 0.39 |
| High Normal Range | 6.16 |
| 70% Confidence Intervals for 2.5 Percentile | |
| Low Range | 0.28-0.53 |
| High Range | 5.60-6.82 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 14.0 PERFORMANCE CHARACTERISTICS

### 14.1 Precision

The within and between assay precision of the Free Thyroid Panel were 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 4 and Table 5 (fT4), Table 6 and Table 7 (fT3) and Table 8 and Table 9 (TSH).

**TABLE 4**
**Within Assay Precision – FT4 Values in ng/dl**

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Low | 24 | 0.925 | 0.057 | 6.2% |
| Normal | 24 | 2.00 | 0.059 | 2.9% |
| High | 24 | 2.93 | 0.071 | 2.4% |

**TABLE 5**
**Between Assay Precision**

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Low | 10 | 0.97 | 0.13 | 13.4% |
| Normal | 10 | 2.06 | 0.09 | 4.4% |
| High | 10 | 2.90 | 0.14 | 4.5% |

*As measured in ten experiments in duplicate over ten days.

**TABLE 6**
**Within Assay Precision – FT3 Values in pg/ml**

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Low | 24 | 2.090 | 0.152 | 7.2% |
| Normal | 24 | 5.308 | 0.222 | 4.2% |
| High | 24 | 9.536 | 0.473 | 5.0% |

**TABLE 7**
**Between Assay Precision**

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Low | 10 | 1.89 | 0.19 | 10.0% |
| Normal | 10 | 5.4 | 0.50 | 9.3% |
| High | 10 | 9.3 | 0.37 | 4.0% |

*As measured in ten experiments in duplicate over ten days.

**TABLE 8**
**Within Assay Precision – TSH values in µIU/ml**

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Pool 1 | 24 | 0.463 | 0.028 | 5.95% |
| Pool 2 | 24 | 5.536 | 0.121 | 2.19% |
| Pool 3 | 24 | 33.109 | 2.061 | 6.23% |

**TABLE 9**
**Between Assay Precision**

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Pool 1 | 24 | 0.445 | 0.042 | 9.4% |
| Pool 2 | 24 | 5.811 | 0.141 | 2.43% |
| Pool 3 | 24 | 35.19 | 3.11 | 4.99% |

*As measured in ten experiments in duplicate over seven days.

### 14.2 Sensitivity

The fT4 procedure has a sensitivity of 0.04 ng/dl. The sensitivity was ascertained by determining the variability of the 0 ng/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

The fT3 procedure has a sensitivity of 0.04 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

The TSH sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose: For I hr incubation = 0.065 µIU/ml

### 14.3 Accuracy

The Free Thyroid Panel AccuBind® ELISA Test System was compared with reference immunometric methods. The least square regression equation and the correlation coefficient were computed for the ELISAs in comparison with the reference methods. The data obtained are displayed in Table 8-10.

**TABLE 10 (fT4)**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| Monobind | 1.38 | y=0.073+0.964(x) | 0.920 |
| Reference | 1.40 | | |
| Range of Values: | | 0.15-9.5 | N=65 |

**TABLE 11 (fT3)**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| Monobind | 3.11 | y=0.11+0.97(x) | 0.985 |
| Reference | 3.20 | | |
| Range of Values: | | 0.80-12.5 | N=65 |

**TABLE 12 (TSH)**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| Monobind | 4.54 | y=0.47+0.968(x) | 0.995 |
| Reference | 4.21 | | |
| Range of Values: | | 0.01-61 | N=65 |

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

### 14.4 Specificity

The cross-reactivity of the antibodies used to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyroid hormone needed to displace the same amount of tracer.

**TABLE 13 - fT4**

| Substance | Cross Reactivity | Concentration |
|---|---|---|
| l-Thyroxine | 1.0000 | - |
| d-Thyroxine | 0.9800 | 10 µg/dl |
| d-Triiodothyronine | 0.0150 | 100 µg/dl |
| l-Triiodothyronine | 0.0300 | 100 µg/dl |
| Iodothyrosine | 0.0001 | 100 µg/ml |
| Diiodothyrosine | 0.0001 | 100 µg/ml |
| Diiodothyronine | 0.0001 | 100 µg/ml |

**TABLE 14 - fT3**

| Substance | Cross Reactivity | Concentration |
|---|---|---|
| l-Triiodothyronine | 1.0000 | - |
| l-Thyroxine | < 0.0002 | 10 µg/ml |
| Iodothyrosine | < 0.0001 | 10 µg/ml |
| Diiodothyrosine | < 0.0001 | 10 µg/ml |
| Diiodothyronine | < 0.0001 | 10 µg/ml |
| Phenylbutazone | < 0.0001 | 10 µg/ml |
| Sodium Salicylate | < 0.0001 | 10 µg/ml |

**TABLE 15 - TSH**

| Substance | Cross Reactivity | Concentration |
|---|---|---|
| Thyrotropin (hTSH) | 1.0000 | - |
| Follitropin (hFSH) | < 0.0001 | 1000 ng/ml |
| Lutropin Hormone (hLH) | < 0.0001 | 1000 ng/ml |
| Chorionic Gonadotropin (hCG) | < 0.0001 | 1000 ng/ml |

## 15.0 REFERENCES


1. Barker, SB, "Determination of Protein Bound Iodine." *Journal Biological Chemistry*, 173, 175 (1948).
1. Chopra, I.J., Solomon, D.H., an Ho, R.S., "A Radioimmunoassay of Thyroxine," *J. Clinical EndocrinoL*, 33, 865 (1971).
2. Young, D.S., Pestaner, L.C., and Gilberman, U., "Effects of Drugs on Clinical Laboratory Tests." *Clinical Chemistry*, 21, 3660 (1975).
3. Sterling, L., *Diagnosis and Treatment of Thyroid Disease*, Cleveland CRC Press, P. 19-51 (1975).
4. Rae P, Farrar J, Beckett G, Toft A, *'Assessment of thyroid status in elderly people.' British Med. Jour* 1993;307:177-180.
5. Charkes ND, *"The many causes of subclinical hyperthyroidism." Thyroid* 1996;6:391-396.
6. Chou FF, Wang PW, Huang SC, *'results of Subtotal Thyroidectomy for Graves' disease.' Thyroid*;9:253-256.
7. Muzzaffari EL, Gharib H, "Thyroxine suppressive therapy in patients with nodular thyroid disease." *Ann Intern Med* 1998; 128:386-394.
8. Attwood EC, Seddon RM, Probert DE: "The T4/TBG ratio and the investigation of thyroid function." *Clin Biochem* 1978; 11:218.
9. Jain R, Isaac RM, Gottschalk ME, et al: 'Transient central hypothyroidism as a cause of failure to thrive in newborns and infants.' *J. Endocrinology Invest.* 1994; 17:631-637.



**Revision: 6 Date: 2022-MAY-25 DCO: 1565**
**MP7025 Product Code: *7025-300***


| Size | | 192(B) | 480(D) |
|---|---|---|---|
| Reagent (fill) | A) | 1ml set | 2 1ml sets |
| | B) | 1 (7ml) | 2 (7ml) |
| | C) | 1 (7ml) | 2 (7ml) |
| | D) | 1 (13ml) | 1 (35ml) |
| | E) | 1 (7ml) | 2 (7ml) |
| | F) | 1 (7ml) | 2 (7ml) |
| | G) | 2 plates | 5 plates |
| | H) | 1 (20ml) | 2 (60ml) |
| | I) | 2 (12ml) | 1 (52ml) |
| | J) | 2 (8ml) | 1 (30ml) |


For Orders and Inquires, please contact

**Monobind Inc.**
100 North Pointe Drive
Lake Forest, CA 92630 USA

Tel: +1 949.951.2665 Mail: info@monobind.com
Fax: +1 949.951.3539 Fax: www.monobind.com

IVD 2° 8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
**www.cepartner4u.eu**


Please visit our website to learn more about our products and services.

### Glossary of Symbols
(EN 980/ISO 15223)