Thyrotropin (TSH) Test System

Product Code: 325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Thyrotropin Concentration in Human Serum by a Microplate Immunoassay method

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 Daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pluri) hypothyroidism (1, 2). The structure of human TSH is similar to that of the pituitary and placental gonadotrophins, consisting of an alpha- and beta-subunit which is similar or identical between these hormones and a 115-amino acid beta-subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with apparent excess production of the alpha-subunit. The TSH molecule has a linear structure consisting of the protein core with carbohydrate chains; the latter accounts for 16% of the molecular weight.

TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary type. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, levels of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoassay methods has provided the laboratory with sufficient sensitivity to enable the differentiating of hypothyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second generation assay, which provides the means for discrimination in the hyperthyroid-euthyroid range. The functional sensitivity (20% between assay CV) of the one-hour procedure is 0.195 µU/ml. All the one-hour procedure has a functional sensitivity of 0.095µU/ml (3).

In this method, TSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from the unbound enzyme-thyrotropin 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 thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve quantitation of an unknown specimen’s activity can be correlated with thyrotropin concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (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, 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:

\[ E_{\text{Ab} +} + A_{\text{Ag}} + A_{\text{Ab}} + A_{\text{Ag}} \rightarrow E_{\text{Ab} +} + A_{\text{Ab}} \rightarrow E_{\text{Ab} +} + A_{\text{Ab}} \]

\[ E_{\text{Ab} +} + A_{\text{Ab}} + A_{\text{Ag}} \rightarrow E_{\text{Ab} +} + A_{\text{Ab}} \]

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

\[ E_{\text{Ab} +} + A_{\text{Ab}} + A_{\text{Ag}} \rightarrow E_{\text{Ab} +} + A_{\text{Ab}} \]

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

4.0 REAGENTS

Materials Provided:

A. Thyrotropin Calibrators – 1mLvial - Icons A-G

- One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinyl monoclonal mouse IgG in a sodium chloride and sodium phosphate buffer. Store at 2-8°C.

B. TSH Enzyme Reagent – 1mLvial - Icon ▶

- One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinyl monoclonal mouse IgG in a sodium chloride and sodium phosphate buffer. Store at 2-8°C.

- Streptavidin Coat Plate – 96 wells - Icon ▶

- One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml - Icon ▶

- One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7mLvial - Icon ▶

- One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7mLvial - Icon ▶

- One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

G. Stop Solution – 1mLvial - Icon ▶

- One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Do not use reagents that are contaminated or have imperfections in a microplate reader.

Note 3: EnzAb

- Subunit which is similar or identical between these hormones and a 115-amino acid beta-subunit, which apparently confers hormonal specificity.

Note 4: Before proceeding with the assay, bring all reagents, serum and specimen to room temperature (20 - 27°C).

**Test Procedure should be performed by a skilled individual or trained professional**

- Format the microwells’ wells for each serum reference, control and patient specimen to be assayed in duplicate.

- Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

- Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.

- Add 0.100 ml (100µl) of the TSH Enzyme Reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.

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

- Incubate 60 minutes at room temperature.

- Discard the contents of the microwell by decantation or aspiration. If decanting, tap and blot the plate dry with filter paper.

- Add 350µl of wash buffer (see Reagent Preparation Section) directly to each well. Gently mix and aspirate. Repeat 2 or 3 additional times for a total of three (3) washes.

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

- DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

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

- Add 0.050ml (50µl) of stop solution to each well and mix gently for 30 seconds.

- Read the absorbance in each well at 450nm (using a microplate reader).

- **For In Vitro Diagnostic Use**

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

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 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 are handled as potentially hazardous in order to prevent inadvertent transmission of disease. Good laboratory procedures for washing, blotting and 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.

6.0 TEST PROCEDURE

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

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

- Add 0.050ml (50µl) of stop solution to each well and mix gently for 30 seconds.

- 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 thirty (30) minutes of adding the stop solution.

- **For better low-end sensitivity (< 0.5µU/ml), incubate 120 minutes at room temperature. The 40µl/µl calibrator should be exchanged for the 20µl/µl calibrator over 3.0 units will be experienced. Follow the remaining steps.

- Dilute samples reading over 40 µL/1.5 and 1.0 with TSH ‘0’ Calibrator. Multiply the results by the dilution factor to obtain accurate results.
10.0  CALCULATION  OF  RESULTS

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

1.  Record  the  absorbance  obtained  from  the  printout  of  the  microplate  reader  as  outlined  in  Example 1.
2.  Plot  the  absorbance  for  each  duplicate  serum  reference  versus  the  corresponding  concentration  in  µIU/ml  on  linear  graph  paper  (do  not  average  the  duplicates  of  the  serum  references  before  plotting).
3.  Draw  the  best-fit  curve  through  the  plotted  points.
4.  To  determine  the  concentration  of  TSH  for  an  unknown,  locate  the  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  µIU/ml)  from  the  horizontal  axis  of  the  graph  (the  duplicates  of  the  unknown  may  be  averaged  as  indicated).

In  the  following  example,  the  average  absorbance  (0.775)  intersects  the  dose  response  curve  at  (7.66  µIU/ml)

The  TSH  concentration  (See  Figure  1).

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

12.0  RISK  ANALYSIS

The  MSDS  and  Risk  Analysis  Form  for  this  product  is  available  on  request  from  Monobind  Inc.

12.1  Assay  Performance

1.  It  is  important  that  the  time  of  reaction  in  each  well  be  held  constant  to  a  specified  range.
2.  Pipetting  of  samples  should  not  extend  beyond  ten  (10)  minutes  to  avoid  assay  drift.
3.  Introduce  the  homogenized  or  grossly  contaminated  specimen(s)  should  not  be  used.
4.  If  more  than  one  (1)  plate  is  recommended  to  repeat  the  assays  for  each  specimen.
5.  The  addition  of  substrate  solution  initiates  a  kinetic  reaction,  which  is  terminated  by  the  addition  of  the  stop  solution.  Therefore,  the  substrate  should  not  be  added  in  the  same  sequence  to  eliminate  any  time-during  reaction  during  development.
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.  Accurate  and  precise  pipetting,  as  well  as  the  following  the  exact  time  and  temperature  requirements  prescribed  are  essential.
10.  Any  deviation  from  Monobind’s  IFU  may  yield  inaccurate  results.
11.  Patient  specimens  with  TSH  concentrations  over  40µIU/ml  may  be  diluted  (1:5  or  1:10)  with  the  ‘0’  calibrator  and  re-assayed.  The  sample's  concentration  is  obtained  by  multiplying  the  result  by  the  dilution  factor.
12.  It  is  important  to  calibrate  all  the  equipment  e.g.  Pipettes,  Readers,  Washers  and/or  the  automated  instruments  used  with  this  device  in  accordance  with  routine  preventative  maintenance.
13.  Analytical  -  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.  Measurement  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.  For  valid  test  results,  accurate  controls  and  other  parameters  must  be  within  the  listed  ranges  and  assay  requirements.
4.  If  test  kits  are  altered,  such  as  mixing  parts  of  different  kits,  which  could  produce  false  test  results,  or  if  results  are  incorrectly  interpreted  for  laboratory  purposes.
5.  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.
6.  Serum  TSH  concentration  is  dependent  upon  a  multiplicity  of  factors:  hypothalamic  gland  function,  thyroid  gland  function,  and  the  response  of  the  pituitary  to  TSH.  Thus,  thyrotropin  concentration  alone  is  not  sufficient  to  assess  clinical  status.
7.  Serum  TSH  values  may  be  elevated  by  pharmacological  intervention.  Dopomiperone,  amiodarone,  iodide,  phenobarbital,  and  phenytoin  have  been  reported  to  increase  TSH  levels.
8.  A  decrease  in  thyrotropin  values  has  been  reported  with  the  administration  of  propranolol,  methimazol,  dopamine  and  thyroxine  (4).
9.  Genetic  variations  or  degradation  of  intact  TSH  into  subunits  may  affect  the  binding  characteristics  of  the  antibodies  and  influence  the  final  result.  Such  samples  normally  exhibit  different  results  among  various  assay  systems  due  to  the  reactivity  of  the  antibodies  involved.

"NOT  INTENDED  FOR  NEWBORN  SCREENING"

13.0  EXPECTED  RANGES  OF  VALUES

A  study  of  euthyroid  adult  population  was  undertaken  to  determine  expected  values  for  the  TSH  AccuBind™  ELISA  Test

System.  The  number  and  determined  range  are  given  in  Table  1.  A  nonparametric  method  (95%  Percentile  Estimate)  was  used.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyrotrpin (hTSH)</td>
<td>&lt;0.001</td>
<td>100ng/ml</td>
</tr>
<tr>
<td>Follicitin (hFSH)</td>
<td>&lt;0.001</td>
<td>100ng/ml</td>
</tr>
<tr>
<td>Lutropin Hormone (hLH)</td>
<td>&lt;0.001</td>
<td>100ng/ml</td>
</tr>
<tr>
<td>Chlorionic</td>
<td>&lt;0.001</td>
<td>100ng/ml</td>
</tr>
</tbody>
</table>

14.5  Correlation  between  1  hr  and  2  hr  incubation

The  one- (1)  and  two  (2)  (optional)  incubation  procedures  were  compared.  Thirty  (30)  biological  specimens  (ranging  from  0.1  –  16.5  µIU/ml)  were  used.  Three  least  square  regression  equation  and  the  correlation  coefficient  were  computed  for  the  2  procedure  (y)  in  comparison  with  the  1  hour  (x)  method.  Excellent  agreement  is  evidenced  by  the  correlation  coefficient,  slope  and  intercept:

\[ Y = 0.986 \times x + 0.119 \]

Regression  Correlation  =  0.998

15.0  REFERENCES

8.  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.

11.9  G.C.  PARAMETERS

In  order  for  the  assay  results  to  be  considered  valid  the  following  criteria  should  be  met:
1.  The  absorbance  of  calibrator  ‘G’  (40  µIU/ml)  should  be  >  1.3.
2.  Four  out  of  six  quality  control  pools  should  be  within  the  established  ranges.

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