3.0 PRINCIPLE

Sequential immunoenzymometric assay Type 4: 
A sequential immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope binding sites. For this procedure, the immobilization takes place during the assay at the stage of biotinylated well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing elution buffer with monoclonal biotinylated antibody added, a complex is formed containing the antigen-antibody reaction between the eluted antigen and the antibody to form an antigen-antibody complex. The interaction is illustrated by the following equation:

\[ Ag(TSH)_{e} + BtnAb(m) = Ag(TSH)_{e}BtnAb(m) \]

3.1 MATERIALS

B. N-TSH Controls – Dried Blood Spots (Two rows by three dots levels - 2 x 6)
C. N-TSH Enzyme Reagent – 13ml vial - 
One (1) vial containing enzyme affinity purified polyclonal goat x-TSH IgG in buffer, and preservative. Store at 2-8°C.
D. N-TSH Biotin Reagent – 13ml vial - 
Anti-TSH monoclonal IgG labeled with biotin in buffer, green dye and preservative. Store at 2-8°C.
E. N-TSH Positive Control - 0.100 ml - Icon I 
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
F. Wash Solution Concentrate – 20 ml/vial - Icon B
One vial containing wash buffer (semble) in 0.05M Tris and hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.
H. Stop Solution – 8ml/vial - Icon C
One (1) vial containing a strong acid (1N H2SO4). Store at 2-8°C.
I. Product Instructions
1. Test Procedure
2. Reagent Preparation
3. Sample Collection and Preparation
4. Safety and Disposal
5. Quality Control
6. Maintenance
7. Storage
8. Troubleshooting

9.1 Alternate overnight procedure
1. Substitute overnight incubation (12-16hrs) for the 90 minutes with rotation (Step 5). No rotator is required. Seal the plate(s) with plastic wrap.
2. All other steps remain the same.

10.0 CALCULATION OF RESULTS
A dose response curve is used to ascertain the concentration of thyropins in unknown specimen.
1. Record the absorbance obtained from the pointut of the microplate reader as outlined in Example 1.
2. Plot the absorbance against the corresponding TSH 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 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 µIU/ml) from the horizontal axis of the graph. In the following example, the average absorbance (0.543) intersects the dose response curve at 66.3 µIU/ml TSH concentration (See Figure 1).

Note: Computer data reductions 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.

12.1 Assay Performance
It is important that the time of reaction in each well is held constant to achieve reproducible results.
1. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
2. Highly ionic, hemolyzed or grossly contaminated serum(s) should not be used.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. The addition of a stop solution initiates a kinetic reaction, terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same proportion to each plate.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate precision in pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
9. All applied standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
10. It is important to calibrate all the equipment e.g. Pipettors, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
11. Re-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. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if test results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is impractical to calibrate the readers for calibrators fall within 10% of the assigned concentrations.
6. TSH concentration, in the circulation, is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone is not sufficient to assess clinical status.
7. TSH values may be elevated by pharmaceutical intervention. Dopamine, 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 d-thyroxine.
9. Genetic variations or degradation of intact TSH into subunits may affect the responsiveness of the anterior pituitary gland and the influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies.

13.0 EXPECTED RANGES OF VALUES
Recommended guidelines for newborn screening for congenital hypothyroidism have been published by the American Academy of Pediatrics (AAP). For infants 2 to 6 days old, these recommendations categorize TSH concentrations as "normal", "elevated", or "only slightly elevated" relative to values of 50 and 40 µIU/ml (i.e. per milliliter of serum). According to the AAP guidelines, any infant with a low TSH and T4 concentration greater than 40 µIU/l is considered to have primary hypothyroidism unless proven otherwise. Furthermore, "in cases in which the TSH is elevated, only a slight elevation is considered only slightly elevated and not to be confused with true primary hypothyroidism."

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision
The within assay precision within assay precisions of the N-TSH Accubind® ELISA test system were determined by analyses on three different levels of pooled whole blood samples. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Tables 2 and 3.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reactivity</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Thyrotropin (N1SH)</td>
<td>&gt; 95%</td>
<td>&lt; 0.0010 ng/mL</td>
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<tr>
<td>Follicin (NFSH)</td>
<td>&gt; 95%</td>
<td>&lt; 0.0010 ng/mL</td>
</tr>
<tr>
<td>Luteinizing Hormone (LH)</td>
<td>&gt; 95%</td>
<td>&lt; 0.0010 ng/mL</td>
</tr>
<tr>
<td>Chorionic Gonadotropin (HCG)</td>
<td>&gt; 95%</td>
<td>&lt; 0.0010 ng/mL</td>
</tr>
</tbody>
</table>

15.0 REFERENCES
6. Centers for Disease Control, "Results of Newborn Screening Programs [Atlanta 1979].

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<table>
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<tr>
<th>Size</th>
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<th>2 Plate</th>
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<tbody>
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<td>480 Test</td>
<td>960 Test</td>
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<td>A)</td>
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</tr>
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<td>2 x 52 ml</td>
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Note: The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.