THYROID STIMULATING HORMONE (TSH) LIA Assay Kit

Catalog Number:
THH31-L01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v 1.0
INTENDED USE
The Eagle Biosciences Thyroid Stimulating Hormone (TSH) LIA Assay Kit is for the direct quantitative determination of TSH in human serum by a chemiluminescence immunoassay (LIA). The Eagle Biosciences Thyroid Stimulating Hormone (TSH) LIA assay kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION
Thyroid stimulating hormone (TSH) is a glycoprotein hormone of 28KD secreted by the anterior pituitary gland. TSH has two subunits, namely α and β. The α subunit of TSH is similar to the α subunit found in the LH, FSH and hCG glycoprotein hormones. The β subunit however, is specific and differs from hormone to hormone. TSH stimulates positively the production of thyroid hormones T4 and T3. Circulating T4 and T3 regulate the TSH secretion by negative feedback. TSH production is also under the positive control of thyrotropin-releasing hormone (TRH), which is secreted by hypothalamus. Measurement of serum TSH is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary hypothyroidism. In primary hypothyroidism, where there is impaired production of thyroid hormones, the TSH level is typically highly elevated. In secondary or tertiary hypothyroidism where the thyroid hormones are low as a consequence of pituitary or hypothalamic lesions, the TSH level is usually low. Further, a sensitive TSH assay is also able to differentiate the hyperthyroidism from the euthyroid population. TSH is typically suppressed to subnormal levels in most hyperthyroidism. It is recommended to assay both TSH and thyroid hormones for the clinical assessment of thyroid status. But if there were to be only one test to be prescribed for thyroid function, TSH would be the test. TSH determinations are also helpful to monitor patients who receive thyroxine replacement therapy.

PRINCIPLE OF THE ASSAY
The principle of this chemiluminescence immunoassay test follows a typical one-step capture or ‘sandwich’ type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for TSH is immobilized onto the microplate and another monoclonal antibody specific for a different region of TSH is conjugated to horse radish peroxidase (HRP). TSH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the luminescence substrate is added and the relative luminescence units (RLUs) are measured in a microtiter plate luminometer. The RLU formation by the enzymatic reaction is directly proportional to the concentration of TSH in the samples. A set of standards is used to plot a standard curve from which the concentration of TSH in patient samples and controls are read.

PROCEDURAL CAUTIONS AND WARNINGS
1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The kit controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges. The performance of this assay is markedly influenced by the correct execution of the washing procedure!
9. The luminescence substrate solutions (A and B) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
10. When dispensing the substrate, do not use pipettes in which these liquids will come into contact with any metal parts.
11. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
12. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
13. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS
1. All the reagents within the kit are calibrated for the determination of TSH in human serum. The kit is not calibrated for the determination of TSH in saliva, plasma or other specimens of human or animal origin. The kit is not indicated for use with neonatal blood spot for newborn screening.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Samples reading higher than the highest calibrator should be reported as such and should not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
5. This kit is intended for research use only and should not be used in diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL
Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS
Avoid direct contact with reagents. In case of contact, wash with plenty of water.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
This assay is a direct system; no specimen pretreatment is necessary.
REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Precision pipettes to dispense 50, 100, 150 and 300 μL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plastic wrap or microplate cover
5. Microplate luminometer

REAGENTS PROVIDED AND PREPARATION
1. Mouse Anti-TSH Antibody-Coated Break-Apart Well Microplate — Ready To Use
   Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

2. Mouse Anti-TSH Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X50
   Contents: Anti-TSH monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   Volume: 0.3 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation of conjugate working solution: Dilute conjugate concentrate 1:50 in assay buffer before use (example: 40 μL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 μL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. TSH Calibrators — Ready To Use
   Contents: 7 vials containing TSH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of TSH. Calibrated against World Health Organization (WHO) 2nd IS 80/558.
   * Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0 μIU/mL</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>Calibrator B</td>
<td>0.15 μIU/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator C</td>
<td>0.5 μIU/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator D</td>
<td>1.5 μIU/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator E</td>
<td>5.0 μIU/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>15.0 μIU/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator G</td>
<td>30.0 μIU/mL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

   Storage: Refrigerate at 2–8°C.
   Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.
4. Control Low & High — Ready To Use
   Contents: Two vials containing TSH in a protein-based buffer with a non-
   mercury preservative. Prepared by spiking buffer with a defined
   quantity of TSH. Refer to vial labels for expected values and
   acceptable ranges.
   Volume: 0.5 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months in unopened vials or as indicated on label. Once
   opened, the standards should be used within 14 days or aliquotted
   and stored frozen. Avoid multiple freezing and thawing cycles.

5. Wash Buffer Concentrate – Requires Preparation X10
   Contents: One bottle containing buffer with a non-ionic detergent and a
   non-mercury preservative.
   Volume: 50 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: Dilute 1:10 in distilled or deionized water before use. If one whole
   plate is to be used dilute 50 mL of the wash buffer concentrate in
   450 mL of water.

6. Assay Buffer
   Contents: One bottle containing protein-based buffer with a non-mercury
   preservative.
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

7. LIA Substrate Reagent A – Requires Preparation
   Contents: One vial containing luminol plus enhancer.
   Volume: 0.8 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: See preparation of LIA working substrate solution.

8. LIA Substrate Reagent B – Requires Preparation
   Contents: One vial containing stabilized peroxide solution.
   Volume: 1.6 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: See preparation of LIA working substrate solution.

9. LIA Substrate Reagent C – Requires Preparation
   Contents: One bottle containing buffer with a non-mercury preservative.
   Volume: 15 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
   Preparation: See preparation of LIA working substrate solution.
PREPARATION OF LIA WORKING SUBSTRATE SOLUTION
In a clean plastic container (glass is not suitable) mix 1 part of LIA substrate reagent A with 2 parts of LIA substrate reagent B and 20 parts of LIA substrate reagent C. This gives the ready to use substrate solution.

If the whole plate is to be used prepare working substrate solution as follows:

Combine 0.7 mL of LIA substrate reagent A with 1.4 mL of LIA substrate reagent B and 14 mL of LIA substrate reagent C. It is suggested to wait at least 30 minutes prior to use after preparation of the working substrate solution. The working substrate solution is stable for up to 8 hours at room temperature. Discard the leftovers.

ASSAY PROCEDURE
Important Notes:
- All reagents must reach room temperature before use.
- Once the procedure has been started, all steps should be completed without interruption to ensure equal elapsed time for each pipetting step.

1. Prepare working solutions of the conjugate, wash buffer and LIA substrate (refer to reagents provided and preparation section).
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 μL of each calibrator, controls and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 μL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Cover the plate and incubate for 60 minutes on a plate shaker (approximately 200 rpm) at room temperature.
6. Wash the wells 5 times, each time with 300 μL of diluted wash buffer per well and on the last washing, tap the plate firmly against absorbent paper to ensure that it is dry. (The use of an automatic washer is recommended.)
7. Pipette 150 μL of LIA working substrate solution into each well. (We recommend using a multichannel pipette.)
8. Measure the RLUs in each well on a microplate luminometer between 10–30 minutes after addition of the substrate.

CALCULATIONS
1. Calculate the mean RLU of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean RLUs on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean RLU of each unknown duplicate.
4. Read the values of the unknowns directly off the calibrator curve.
5. If a sample reads more than 30 μIU/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.
TYPICAL TABULATED DATA**
Sample data only. Do not use to calculate results.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>RLU 1</th>
<th>RLU 2</th>
<th>Mean RLU</th>
<th>RLU/RLUMAX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 0 μIU/mL</td>
<td>7200</td>
<td>5789</td>
<td>6495</td>
<td>0.37</td>
</tr>
<tr>
<td>B, 0.15 μIU/mL</td>
<td>10337</td>
<td>9346</td>
<td>9842</td>
<td>0.57</td>
</tr>
<tr>
<td>C, 0.5 μIU/mL</td>
<td>21485</td>
<td>20066</td>
<td>20776</td>
<td>1.2</td>
</tr>
<tr>
<td>D, 1.5 μIU/mL</td>
<td>87315</td>
<td>79974</td>
<td>83645</td>
<td>4.8</td>
</tr>
<tr>
<td>E, 5 μIU/mL</td>
<td>265814</td>
<td>270623</td>
<td>268219</td>
<td>15.5</td>
</tr>
<tr>
<td>F, 15 μIU/mL</td>
<td>871495</td>
<td>901500</td>
<td>886498</td>
<td>51</td>
</tr>
<tr>
<td>G, 30 μIU/mL</td>
<td>1614105</td>
<td>1850103</td>
<td>1732104</td>
<td>100</td>
</tr>
</tbody>
</table>

** It is recommended to use the RLU/RLUMAX values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show quite different RLU values, however, the RLU/RLUMAX values remain consistent.

TYPICAL CALIBRATOR CURVE
Sample curve only. Do not use to calculate results.

PERFORMANCE CHARACTERISTICS
SENSITIVITY
The limit of detection (LoD) was determined from the analysis of 40 samples of the blank and a low value sample and it was calculated as follows:

\[
LoD = \mu_B + 1.645\sigma_B + 1.645 \sigma_S, \text{ where } \sigma_B \text{ and } \sigma_S \text{ are the standard deviation of the blank and low value sample and } \mu_B \text{ is the blank concentration result.}
\]

\[
LoD = 0.13 \text{ μIU/mL.}
\]
SPECIFICITY (CROSS-REACTIVITY)
The specificity of the TSH LIA kit was determined by measuring the apparent TSH values of the following compounds:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration Range</th>
<th>Apparent TSH Value (μIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG Calibrated against WHO 1st IS 75/537</td>
<td>10,000-50,000 IU/L</td>
<td>&lt; 0.15</td>
</tr>
<tr>
<td>FSH Calibrated against WHO 1st 83/575</td>
<td>1000-4000 IU/L</td>
<td>&lt; 0.15</td>
</tr>
<tr>
<td>LH Calibrated against WHO 2nd IS 80/552</td>
<td>100-500 IU/L</td>
<td>&lt; 0.15</td>
</tr>
</tbody>
</table>

INTRA-ASSAY PRECISION
Three samples were assayed ten times each on the same calibrator curve. The results (in μIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.009</td>
<td>5.631</td>
</tr>
<tr>
<td>2</td>
<td>1.01</td>
<td>0.057</td>
<td>5.650</td>
</tr>
<tr>
<td>3</td>
<td>11.52</td>
<td>0.989</td>
<td>8.590</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION
Three samples were assayed ten times over a period of four weeks. The results (in μIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.178</td>
<td>0.019</td>
<td>10.68</td>
</tr>
<tr>
<td>2</td>
<td>1.339</td>
<td>0.105</td>
<td>7.84</td>
</tr>
<tr>
<td>3</td>
<td>10.10</td>
<td>0.978</td>
<td>9.68</td>
</tr>
</tbody>
</table>

RECOVERY
Spiked samples were prepared by adding an exact amount of TSH to four patient serum samples. The results (in μIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked + 3.16</td>
<td>1.07</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2 Unspiked + 0.1</td>
<td>0.08</td>
<td>-</td>
<td>88.9</td>
</tr>
<tr>
<td>3 Unspiked + 1.0</td>
<td>1.01</td>
<td>0.18</td>
<td>93.5</td>
</tr>
<tr>
<td>4 Unspiked + 10.0</td>
<td>11.52</td>
<td>10.08</td>
<td>114.3</td>
</tr>
<tr>
<td>3 Unspiked + 3.07</td>
<td>3.71</td>
<td>4.65</td>
<td>80.0</td>
</tr>
<tr>
<td>4 Unspiked + 5.26</td>
<td>5.62</td>
<td>6.84</td>
<td>82.2</td>
</tr>
<tr>
<td>4 Unspiked + 6.51</td>
<td>6.49</td>
<td>8.09</td>
<td>80.2</td>
</tr>
<tr>
<td>4 Unspiked + 6.51</td>
<td>6.62</td>
<td>7.75</td>
<td>85.4</td>
</tr>
</tbody>
</table>
LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in μIU/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:2</td>
<td>1.86</td>
<td>2.08</td>
<td>89.4</td>
</tr>
<tr>
<td>1:4</td>
<td>1.08</td>
<td>1.04</td>
<td>103.8</td>
</tr>
<tr>
<td>1:8</td>
<td>0.56</td>
<td>0.52</td>
<td>107.7</td>
</tr>
<tr>
<td>2</td>
<td>3.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:2</td>
<td>1.56</td>
<td>1.54</td>
<td>101.3</td>
</tr>
<tr>
<td>1:4</td>
<td>0.85</td>
<td>0.77</td>
<td>110.4</td>
</tr>
<tr>
<td>1:8</td>
<td>0.47</td>
<td>0.39</td>
<td>120.5</td>
</tr>
<tr>
<td>3</td>
<td>11.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1:2</td>
<td>5.44</td>
<td>5.53</td>
<td>97.6</td>
</tr>
<tr>
<td>1:4</td>
<td>2.98</td>
<td>2.76</td>
<td>108.0</td>
</tr>
<tr>
<td>1:8</td>
<td>1.74</td>
<td>1.38</td>
<td>126.1</td>
</tr>
</tbody>
</table>

COMPARATIVE STUDIES

The TSH LIA assay was compared to the DBC TSH ELISA method on 20 samples (concentration range: 0.64–5.56 μIU/mL).

By linear regression:

(This method) = 0.92 x (DBC’s ELISA) + 0.14 μIU/mL, r = 0.986

Means:

LIA: 1.87 μIU/mL
ELISA: 2.03 μIU/mL

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (μIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.3–5</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>&lt; 0.15</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>&gt; 5.7</td>
</tr>
</tbody>
</table>

REFERENCES

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.