**INTENDED USE**

The thyroid stimulating hormone (thyrotropin or TSH) is a glycoprotein with a molecular weight of 28 kDa, secreted by the adenohypophysis.

The synthesis and the release of TSH are controlled by the circulatory level of thyroid hormones triiodothyronine (T₃) and thyroxin (T₄) and by the hypothalamic thyrotropin releasing hormone (TRH).

Thyroid hormones regulate the secretion of TSH by a negative feedback mechanism. An elevation of T₃ or T₄ will suppress, and their decrease will, in turn, increase the level of TSH in serum. The increased concentration of TSH in the serum is the earliest and best indicator of primary hypothyroidism.

**PRINCIPLE of the TEST**

The Eagle Biosciences TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay Kit is a one-step enzyme immunoassay (sandwich). The HRP-conjugated antibody (signal) and the capture antibody coated on the plate react simultaneously with the TSH (antigen) present in standards or samples.

After an incubation of 1 hour, a specific sandwich complex (capture antibody-TSH-conjugated antibody) is resulting immobilised on the surface of the wells. The unbound fraction will be removed by washing steps.

The substrate solution is added into each well for colour development. The intensity of the colour in the well is proportional to the concentration of TSH in serum.

Optical density (OD) should be measured at 450 nm and 405 nm. With the obtained values two standard curves are elaborated (concentration versus OD): A) concentration of calibrators 0-5 (0-2.5 µIU/ml) versus OD at 450 nm; B) Concentration of calibrators 4-7 (0.75-15 µIU/ml) versus OD at 405 nm. TSH concentrations of the unknown samples must be read-off as follow: samples with an expected TSH concentration between 0 and 2.5 µIU/ml on curve at 450 nm; samples with an expected TSH concentration between 2.5 and 15.0 µIU/ml on curve at 405 nm.

If expected TSH concentration in the sample is higher than calibrator 7 (15 µIU/ml) samples should be diluted 10-fold with calibrator 0 (0 µIU/ml).

**SAMPLES**

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Do not use plasma, lipaemic or haemolytic samples, or serum containing sodium azide.

The samples may be kept at 2 - 8 °C for up to two days. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep them at -20 °C.
A microtiter plate is in a reclosable foil bag. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original bag carefully resealed.

B) Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer 10 times (1 + 9) with distilled water. For example, dilute 10 ml of the concentrate with 90 ml of distilled water. The washing solution prepared is stable up to 30 days at 2 - 8 °C or.

Cl, CII) D) E) store at 2 - 8 °C for not more than 4 weeks after opening. 0, 1-7) store at 2 - 8 °C for not more than 4 weeks after opening.

Crystallisation of undiluted wash buffer may occur. Crystals can be dissolved by warming up at 37 °C. Avoid exposure of the TMB substrate solution to light!

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 0 - 7 on the ordinate, y-axis versus their respective TSH concentrations on the abscissa, x-axis.

TSH concentrations of the unknown samples are directly read off in µIU/ml against the respective OD values.

TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay Kit may be used also with Computer Assisted Analysis using software able to calculate curves with spline smoothing fit.

TYPICAL EXAMPLE

Do not use for evaluation!

<table>
<thead>
<tr>
<th>Sample</th>
<th>OD (a) 450 nm</th>
<th>OD (b) 450 nm</th>
<th>OD (mean)</th>
<th>µIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 0</td>
<td>0.014</td>
<td>0.013</td>
<td>0.014</td>
<td>0.000</td>
</tr>
<tr>
<td>Calibrator 1</td>
<td>0.025</td>
<td>0.025</td>
<td>0.025</td>
<td>0.020</td>
</tr>
<tr>
<td>Calibrator 2</td>
<td>0.059</td>
<td>0.056</td>
<td>0.058</td>
<td>0.075</td>
</tr>
<tr>
<td>Calibrator 3</td>
<td>0.160</td>
<td>0.157</td>
<td>0.158</td>
<td>0.250</td>
</tr>
<tr>
<td>Calibrator 4</td>
<td>0.454</td>
<td>0.439</td>
<td>0.446</td>
<td>0.750</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>1.439</td>
<td>1.428</td>
<td>1.434</td>
<td>2.500</td>
</tr>
<tr>
<td>Control CI</td>
<td>0.726</td>
<td>0.728</td>
<td>0.727</td>
<td>1.345</td>
</tr>
</tbody>
</table>

The above mentioned standard concentrations are only an example for a typical standard curve. They can change from lot to lot.

<table>
<thead>
<tr>
<th>Sample</th>
<th>OD (a) 405 nm</th>
<th>OD (b) 405 nm</th>
<th>OD (mean)</th>
<th>µIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator 4</td>
<td>0.141</td>
<td>0.139</td>
<td>0.140</td>
<td>0.750</td>
</tr>
<tr>
<td>Calibrator 5</td>
<td>0.428</td>
<td>0.429</td>
<td>0.428</td>
<td>2.500</td>
</tr>
<tr>
<td>Calibrator 6</td>
<td>1.128</td>
<td>1.116</td>
<td>1.122</td>
<td>7.500</td>
</tr>
<tr>
<td>Calibrator 7</td>
<td>1.934</td>
<td>1.896</td>
<td>1.915</td>
<td>15.000</td>
</tr>
<tr>
<td>Control CI</td>
<td>0.219</td>
<td>0.222</td>
<td>0.220</td>
<td>1.285</td>
</tr>
</tbody>
</table>

Materials required

- Precision pipettes 10 - 100 µl
- Multi-channel pipette 300 µl
- Disposable pipette tips
- 8-channel wash comb with a vacuum pump or microtiter plate washer
- Microtiter plate reader with optical filters for 450 nm, 405 nm (or 492 nm alternatively) and 620 nm (or 690 nm alternatively)
- Graduated cylinders
- Distilled water
- Absorbent paper or paper towel
- Microplate shaker-thermostat (able to maintain a temperature of +37 °C and a shaking speed of up to 700 rpm)

Size and storage

TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay Kit has been designed for 96 determinations. This is sufficient for the analysis of 38 unknown samples as well as for calibrators and the control serum, all assayed in duplicates.

The expiry date of each component is recorded on its respective label; that of the complete TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA kit is printed on the box labels.

Upon receipt, all components of the TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA have to be kept at 2 - 8 °C, preferably in the original TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA box.

Preparation before use

Allow all components to reach room temperature prior to use in the assay for at least 30 minutes. Take care to agitate serum samples gently in order to ensure homogeneity.
ASSAY PROCEDURE

1. Bring all reagents to room temperature before use. Mix gently, avoid foam.

2. Dispense 50 µl calibrators (0 - 7) and 50 µl control sera (CI, CII) into the respective wells.

3. Add 100 µl of conjugate (D) to each well.

4. Cover the plate and incubate for 1 hour at 37 °C while shaking at 700 rpm.

5. Aspirate and wash the wells 5 times with 300 µl of washing solution (made from B). Strike the wells sharply onto absorbent paper towels to remove all residual water droplets.

6. Add 100 µl of substrate (E) to each well.

7. Cover the plate and incubate 15 min in the dark at 37 °C while shaking at 700 rpm.

8. Add 100 µl of stop solution (F) to each well and shake 5 sec. (be sure that all the blue colour changes to yellow colour completely).

9. Read the optical density at 450 nm (0 - 7.5 µIU/ml) and 405 nm or 492 nm (up to 15.0 µIU/ml) versus 620 nm or 690 nm within 20 min after adding the stop solution.

Standardization

The included TSH standard curve is calibrated against the actual International Reference Preparation: 2nd IRP 80/558

Criteria of validation

Specimens with an expected TSH concentration between 0 and 2.5 µIU/ml should be read-off on curve at 450 nm; samples with an expected TSH concentration between 2.5 and 15.0 µIU/ml on curve at 405 nm.

REFERENCE VALUES

According to the Guidelines Development of the National Academy of Clinical Biochemistry (NACB) all laboratories should establish their own reference interval independent of the manufacturer’s recommendations. According to this, the following reference values should be for orientation only.

In normal, healthy persons, the serum TSH values range from 0.3 to 3.5 µIU/ml.

In clinically euthyroid samples, serum TSH is normally below 3.5 µIU/ml. This applies also to samples on thyroid hormone replacement or on and after antithyroid drug treatment, respectively. Furthermore, samples suffering from severe non-thyroidal illness (NTI) etc., show frequently subnormal TSH values.

Sub-clinical hypothyroidism is associated with basal TSH values above 3 µIU/ml whereas serum TSH above 5 µIU/ml is more and more indicative for overt hypothyroidism.

Subnormal TSH values (< 0.3 µIU/ml) are observed in sub-clinical hyper-thyroidism. In thyrotoxicosis, however, the TSH is suppressed (< 0.1 µIU/ml).

CHARACTERISTIC ASSAY DATA

Sensitivity

The analytical sensitivity is 0.007 µIU/ml calculated as mean (n = 12) of a TSH free serum + 3 SD.

The functional sensitivity is determined to be 0.015 µIU/ml (20 % Inter-assay CV):

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>mean (µIU/ml)</th>
<th>CV (%)</th>
<th>Sample no.</th>
<th>mean (µIU/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>4</td>
<td>5</td>
<td>0.02</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>5</td>
<td>6</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>3</td>
<td>7</td>
<td>3.4</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>5.6</td>
<td>1</td>
<td>8</td>
<td>12.8</td>
<td>2</td>
</tr>
</tbody>
</table>

Specificity

The following hormones were tested: hCG, LH, FSH and no cross reactivity was detected. For TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA kit **high dose hook effect** was not detected for concentrations up to 800 µIU/ml.
# TSH Ultrasensitive ELISA Assay Kit

## ASSAY SCHEME

| Step | Activity                  | Material                                | CAL            | Control sera | Samples 1, 2 etc.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bring to room temperature</td>
<td>All test components</td>
<td>CAL 0 - 7</td>
<td>C1, CII</td>
<td>1, 2, ...</td>
</tr>
<tr>
<td>2</td>
<td>Pipette</td>
<td>Calibrators (0-7) Control (C) Samples</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>3</td>
<td>Pipette</td>
<td>Conjugate (D)</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>4</td>
<td>Cover and incubate</td>
<td></td>
<td>1 hour at 37 °C with shaking (700 rpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Aspirate or decant</td>
<td>Washing solution (made from B)</td>
<td>5 x 300 µl</td>
<td>5 x 300 µl</td>
<td>5 x 300 µl</td>
</tr>
<tr>
<td></td>
<td>Pipette</td>
<td></td>
<td>put sharply onto absorbent tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspirate or decant</td>
<td></td>
<td>put sharply onto absorbent tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Pipette</td>
<td>Substrate (E)</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>7</td>
<td>Cover and incubate</td>
<td></td>
<td>15 min at 37 °C while shaking in the dark (700 rpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Pipette and shake</td>
<td>Stop solution (F)</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>9</td>
<td>Measure OD</td>
<td></td>
<td>at 450 nm and 405 nm (or 492 nm) versus 620 nm (or 690 nm) within 20 min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## SAFETY PRECAUTIONS

- **This TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay kit is for research use only.** Follow the working instructions carefully. This instruction manual is valid only for the present TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels of the **TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay kit** are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents of the TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay kit from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents of the TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay kit should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Kathon MW as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
• Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

• Since the TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

In any case GLP should be applied with all general and individual regulations to the use of this TSH Ultrasensitive (Thyroid Stimulating Hormone) ELISA Assay kit.

**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.*