The monoclonal anti-TSH antibody coated on the surface of the microwells specifically recognizes the junction between the α and β subunits. The HRP-conjugated monoclonal anti-TSH antibody detects the β subunit only.

During the assay, the test specimen and HRP-anti-TSH conjugates are incubated simultaneously with the coated microwells. The TSH, if present in the specimen, reacts to the anti-TSH antibody coated on the microwell surface as well as the HRP-anti-TSH conjugates, forming an antibody sandwich immunocomplex.

Unbound conjugates are then removed by washing. The presence of the conjugate complex is shown by development of a blue color upon additional incubation with substrate. The reaction is terminated with Stop Solution and the absorbance determined using a spectrophotometer at 450/620-690 nm.

A standard curve is generated by plotting the absorbance at 450/620-690 nm wavelength versus the respective TSH concentration for each standard. The concentration of TSH in the samples is then determined directly from this curve.

**MATERIALS AND REAGENTS**

**Materials and reagents provided with the kit**

**Item** | Description | Quantity | Catalog
--- | --- | --- | ---
1 | Anti-TSH Ab Coated Microwells | 8 wells x 12 strips | E1030W
2 | TSH Standards: S1 (0 µIU/mL) | 1 mL | E1030S1
3 | S2 (0.5 µIU/mL) | 0.75 mL | E1030S2
4 | S3 (2 µIU/mL) | 0.75 mL | E1030S3
5 | S4 (5 µIU/mL) | 0.75 mL | E1030S4
6 | S5 (10 µIU/mL) | 0.75 mL | E1030S5
7 | S6 (20 µIU/mL) | 0.75 mL | E1030S6
8 | S7 (40 µIU/mL) | 0.75 mL | E1030S7
9 | HRP-anti-TSH Conjugates | 6 mL | E1030H
10 | TMB Substrate | 12 mL | TME2001
11 | Wash Buffer (30X Concentrate) | 20 mL | WE3001
12 | Stop Solution | 13 mL | SE1001
13 | Product Insert | 1 set | PI-E1030
14 | ELISA Working Sheet | 1 | E0001ES

Others 2 x Microparticle Seals and 1 x Zip-lock Bag

**Materials and reagents required but not provided in the kit**

1. Pipette capable of delivering 50 µL, 100 µL, 200 µL, and 1 mL with a precision better than 98.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450/620-690 nm wavelength is acceptable.
3. Vortex mixer or equivalent.
4. Absorbent paper for blotting the microwells.
5. Graphic paper
6. Timer
7. Distilled or de-ionized water

**STORAGE AND STABILITY**

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Reroute the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Place unused wells in the zip-lock bag provided and return to 2-8°C. Open vial stability from this point is 2 months at 2-8°C, or until the labeled expiration date, whichever is earlier.

**WARNING AND PRECAUTIONS**

**For In Vivo Diagnostic Use**

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (20-25°C) before use.
4. Do not use the components of any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimens for testing.

**TEST PRINCIPLE**

The RecombiLISA TSH ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of antibody sandwich technique for the quantitative determination of TSH in human serum.

The RecombiLISA TSH ELISA Kit is composed of two key components:

1. Solid microwells pre-coated with monoclonal anti-TSH antibody.
2. Liquid conjugates composed of monoclonal anti-TSH antibody conjugated with horseradish peroxidase (HRP-anti-TSH conjugates).

6. Do not ingest the reagents. Avoid contact with eyes, skin and mucose.

**INTENDED USE**

The RecombiLISA TSH ELISA Kit is a solid phase enzyme linked immunosorbent assay for the quantitative determination of the levels of Thyroid Stimulating Hormone (TSH) in human serum. It is intended to be used by professionals as an aid in the diagnosis of thyroid dysfunction. Any interpretation or use of this test result must also rely on other clinical findings as well as on the professional judgment of health care providers. Alternative test method(s) may be considered to confirm the result obtained by this test.

**INTRODUCTION**

Human thyroid stimulating hormone (TSH) is synthesized by the basophilic cells (thyrotropes) of the anterior pituitary. It is composed of two noncovalently linked subunits designated α and β. The structure of the subunit is similar to that of luteinizing hormone (LH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (HCG). The β subunit is hormone specific and confers biological as well as immunological specificity. Both the α and β subunits are required for its biological activity. TSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3). T4 and T3 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development as well as metabolic and neural activity.

The hypothalamic-pituitary-thyroid (HPT) axis is established by communication and feedback between the hypothalamus, the pituitary gland, and the thyroid. The synthesis and secretion of TSH from the anterior pituitary is stimulated by the hypothalamic tripeptide thyrotropin releasing hormone (TRH), which is released from the hypothalamus in response to low levels of circulating T4/T3. When the hypothalamus senses that T4/T3 hormones have returned to normal levels, TRH and TSH release are inhibited. The hypothalamus may also inhibit TSH release in the event of somatostatin and dopamine. Failure at any level of regulation of the HPT axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of T4 and T3. Recent data from large population studies have shown that mean the TSH level in the general population is approximately 1.50 μIU/mL (95% confidence interval 1.46-1.54 μIU/mL). In the United States, hypothyroidism occurs in about 4.6% of the adult population, and hyperthyroidism is present in 1.3% of population. Most physicians consider TSH levels > 5-10 µIU/mL as evidence of mild or subclinical hypothyroidism, and TSH levels < 0.2-0.4 µIU/mL as evidence of hyperthyroidism. However, it is important for each facility to establish its own TSH reference interval based on representative sampling of the local population.

**SPECIMEN COLLECTION AND PREPARATION**

1. Bring all reagents, controls to room temperature (20-25°C).
2. Mix each reagent before adding to the test wells.
3. If a specimen is not tested immediately, refrigerate at 2-8°C. If storage period greater than three days is anticipated, the specimen should be frozen (−20°C). Avoid repeated freeze-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of biohazardous agents.
4. Do not use serum specimens demonstrating gross lипemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.
5. Serum should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
6. Most physicians consider TSH levels > 5-10 µIU/mL as evidence of mild or subclinical hypothyroidism, and TSH levels < 0.2-0.4 µIU/mL as evidence of hyperthyroidism.
7. If a specimen is not tested immediately, refrigerate at 2-8°C. If storage period greater than three days is anticipated, the specimen should be frozen (−20°C). Avoid repeated freeze-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of biohazardous agents.
8. Do not use serum specimens demonstrating gross lипemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.
9. Do not use serum derived from hemolyzed blood specimens for testing.

**PREPARATION OF THE REAGENTS**

1. Dilute samples with expected TSH concentration over 40 µIU/mL with appropriate concentration of TMB Substrate.
2. Select appropriate concentration of TMB Substrate.
3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA working sheet with the appropriate information. Standards should be run in duplicate to ensure accuracy.
5. Dilute samples with expected TSH concentration over 40 µIU/mL with appropriate concentration of TMB Substrate.
6. Most physicians consider TSH levels > 5-10 µIU/mL as evidence of mild or subclinical hypothyroidism, and TSH levels < 0.2-0.4 µIU/mL as evidence of hyperthyroidism.
Concentration
SD
N
%CV
Mean
%CV

<table>
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<th>Panel</th>
<th>Low</th>
<th>Mean (µIU/mL)</th>
<th>SD</th>
<th>%CV</th>
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<td>0.413</td>
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</table>

1. **Sensitivity**
   - Analytical sensitivity is defined as the concentration corresponding to the mean of 20 replicates of standard 51. Result is presented as average of 3 lot's sensitivity at 95% confidence level: 0.035 ± 0.012 µIU/mL.

2. **Specificity**
   - Specificity was determined by measuring cross reactivity of the RecombiLISA TSH ELISA kit with related hormones hCG, FSH, and LH.

3. **Accuracy**
   - In a total of 156 specimens, TSH levels were measured by Roche E601 TSH Chemiluminescent Immunoassay and RecombiLISA TSH ELISA kit.

4. **Precision**
   - a. Intra-assay precision was determined by assaying 20 replicates of 4 patient pools.
      - Results of a typical standard curve are shown below:

5. **Hook effect**
   - No hook effect was observed at the TSH concentration up to 8,000 µIU/mL.

6. **Interference**
   - Common substances (such as pain and fever medication and blood components) may affect the performance of the RecombiLISA TSH ELISA Kit. This was studied by spiking these substances into pooled human serum. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the RecombiLISA TSH ELISA Kit.

### QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to check on assay performance. Control containing sodium azide cannot be used. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

### LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when assaying the levels of TSH in serum specimens from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The RecombiLISA TSH ELISA kit is limited to the quantitative detection of TSH in serum.
3. The test is for in vitro diagnostic use only.
4. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
5. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

### REFERENCES