**Thyroxine (T₄) ELISA**

Catalog Number SE120121
Storage Temperature 2–8 °C

**Product Description**

Thyroxine (T₄) is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. The level of T₄ is decreased in hypothyroid patients and is increased in hyperthyroid patients. The level of T₄ is normal in euthyroid individuals.

The Thyroxine (T₄) ELISA is intended for the detection of Total T₄ in human serum or plasma. It is a solid phase competitive ELISA. The samples, working T₄-HRP Conjugate and Anti-T₄-Biotin Solution are added to the wells coated with Streptavidin. T₄ in the serum competes with a T₄ enzyme (HRP) conjugate for binding sites. Unbound T₄ and T₄ enzyme conjugate are washed off. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of T₄ in the samples. A standard curve is prepared relating color intensity to the concentration of the T₄.

**Components**

<table>
<thead>
<tr>
<th>Materials Provided</th>
<th>96 Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with Streptavidin</td>
<td>12 x 8 x 1</td>
</tr>
<tr>
<td>T₄ Standard: 6 vials (ready to use)</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Anti- T₄-Biotin Solution</td>
<td>7 mL</td>
</tr>
<tr>
<td>T₄-HRP Conjugate</td>
<td>0.7 mL</td>
</tr>
<tr>
<td>Conjugate Buffer</td>
<td>7 mL</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 mL</td>
</tr>
<tr>
<td>20x Wash concentrate: 1 bottle</td>
<td>25 mL</td>
</tr>
</tbody>
</table>

**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

**Preparation Instructions**

**Sample Preparation**

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (–20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

**T₄-HRP Conjugate Solution**

Dilute the T₄-HRP conjugate 11-fold with Conjugate Buffer in a suitable container. For example, dilute 160 µL of T₄-HRP conjugate with 1.6 mL of Conjugate Buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay.

Store at 2–8 °C.

**Note:**

Volume of Buffer required = Number of wells x 0.1 mL
Volume of Enzyme conjugate solution necessary = number of wells x 0.01 mL
For 16 wells, 16 x 0.1 = 1.6 mL for Total Conjugate Buffer
16 x 0.01 = 0.16 mL (160 µL) for enzyme conjugate solution.

20x Wash Buffer Concentrate

Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

**Storage/Stability**

Store the kit at 2–8 °C.
Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control, and serum samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18–26 °C).

1. Format the microplate wells for each serum reference, control, and specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal, and store at 2–8 °C.

2. Pipette 25 µL of the standards, control or specimen into the assigned well.

3. Add 50 µL of the working T4-HRP conjugate solution to all wells (see Preparation Instructions).

4. Add 50 µL of T4-Antibody-Biotin Solution to all wells.

5. Swirl the microplate gently for 20–30 seconds to mix the reagents.

6. Cover and incubate 60 minutes at room temperature.

7. Remove liquid from all wells. Wash wells three times with 300 µL of 1x Wash Buffer (see Preparation Instructions). Blot on absorbent paper towels.

8. Add 100 µL of TMB substrate solution to all wells.

9. Cover the plate and incubate at room temperature for 15 minutes.

10. Add 50 µL of Stop Solution to each well and gently mix for 15–20 seconds.

11. Read the absorbance on ELISA Reader for each well at 450 nm within 15 minutes after adding the Stop Solution.

Results
The standard curve is constructed as follows:

1. Check T4 standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.

2. To construct the standard curve, plot the absorbance for T4 standards (vertical axis) versus T4 standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

<table>
<thead>
<tr>
<th>OD (450 nm)</th>
<th>Concentration (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Std 1</td>
<td>2.18</td>
</tr>
<tr>
<td>Std 2</td>
<td>1.50</td>
</tr>
<tr>
<td>Std 3</td>
<td>1.19</td>
</tr>
<tr>
<td>Std 4</td>
<td>0.86</td>
</tr>
<tr>
<td>Std 5</td>
<td>0.65</td>
</tr>
<tr>
<td>Std 6</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Expected values
A study of euthyroid in an adult population was undertaken to determine expected values for the T4 EIA test system. The mean value, standard deviation and expected ranges of samples are presented in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Male (42 samples)</th>
<th>Female (58 samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.6</td>
<td>8.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>Expected range</td>
<td>4.4–10.8</td>
<td>4.8–11.6</td>
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</tbody>
</table>
References


