Human Thyroglobulin EIA

For the quantitative determination of human thyroglobulin (hTG) in plasma and serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 30-7510
Size: 96 Wells
Version: 100205 – ALPCO 08/22/06

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1. INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of human thyroglobulin (hTG) in plasma and serum. It is for research use only.

2. INTRODUCTION

Human Thyroglobulin (hTG) is a large glycoprotein (MW 660,000) that is stored in the follicular colloid of the thyroid gland. It functions as a prohormone in the intra-thyroid synthesis of T3 and T4. Traditionally circulating levels of hTG have been measured using double antibody immunoassays. HTG is evaluated in thyroid follicular and papillary carcinoma, thyroid adenoma, subacute thyroiditis, Hashimoto’s thyroiditis and Grave’s disease. HTG levels are not increased in patients with medullary thyroid carcinoma. Low hTG concentrations are an indication that thyrotoxicosis factitia may be present. Measurement of hTG is most useful in detecting recurrence of differentiated thyroid carcinoma following surgical resection or radioactive iodine ablation. HTG determination is used as an adjunct to iodine 131 scanning but not as a replacement for it. Assessment of serum hTG also aids in the management of infants with congenital hypothyroidism.

Indications:
- Postoperative monitoring of differentiated thyroid carcinoma
- Congenital hypothyroidism
- Hyperthyreosis factitia
- Tumor recurrence in patients with thyroid carcinoma
### 3 MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Catalogue No</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 7510MTP</td>
<td>PLATE</td>
<td>One holder with precoated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K 7510WP</td>
<td>WASHBUF</td>
<td>ELISA wash buffer concentrate 10x</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>K 7510K</td>
<td>CONJ</td>
<td>Conjugate, rabbit-anti-hTG antibodies, peroxidase-labeled, ready to use</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>K 7510KO</td>
<td>CTRL</td>
<td>Control, ready-to-use</td>
<td>2 vials</td>
</tr>
<tr>
<td>K 7510ST</td>
<td>STD</td>
<td>Standards, ready to use (0; 4; 8; 16; 31; 62; 125; 250 µg/l)</td>
<td>2 x 8 vials</td>
</tr>
<tr>
<td>K 7510TMB</td>
<td>SUB</td>
<td>Tetramethylbenzidin (TMB) substrate solution, ready to use</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>K 7510AC</td>
<td>STOP</td>
<td>ELISA stop solution, ready to use</td>
<td>1 x 7 ml</td>
</tr>
<tr>
<td>K 7510AP</td>
<td>ASYBUF</td>
<td>Assay buffer, ready to use</td>
<td>1 x 10 ml</td>
</tr>
</tbody>
</table>

### 4 MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water
- Laboratory balance
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm (reference wave length 620 or 690 nm)
5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, make sure that the reagents are stored at the conditions stated on the label. **Prepare just the appropriate amount necessary for the assay.** The kit can be used up to 4 times within the expiry date stated on the label.

- Reagents with a volume less than 100 µl should be centrifuged before use to avoid loss of volume.

- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted with distilled water. 1:10 before use (100 ml concentrate + 900 ml distilled water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals have to be redissolved at room temperature or at 37°C using a water bath before dilution. The **buffer concentrate** is stable at 2-8°C until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at 2-8°C for one month.

- The **standards and the control** can be stored at 2-8°C for 4 weeks. Long term storage until the expiry date given on the label at –20°C only.

- All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

6. SAMPLE PREPARATION

**Serum and plasma samples**

Fresh collected blood should be centrifuged within one hour. Store samples at -20 °C if not assayed on the same day. Lipemic or hemolytic samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicate analyses for each sample.

The **serum** and **plasma** samples should be diluted 1:10 with assay buffer if their hTG concentration is higher than 250 µg/L.

For example:

50 µl sample + 450 µl assay buffer

This protocol is for reference purposes only. DO NOT use this copy to run your assay; use the protocol included with the kit ONLY.
7. ASSAY PROCEDURE

**Principle of the test**

The assay utilizes the “sandwich” technique with two selected polyclonal antibodies that bind to human Thyroglobulin.

Standards, controls and patient samples which are assayed for human thyroglobulin are added into the wells of a microplate coated with a high affine polyclonal anti-human thyroglobulin antibody. During the first incubation step, thyroglobulin is bound by the immobilized antibody. Then a peroxidase-conjugated polyclonal rabbit anti-human thyroglobulin antibody is added into each microtiter well and a “sandwich” of capture antibody - human thyroglobulin – peroxidase-conjugate is formed. Tetramethylbenzidine (TMB) is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of thyroglobulin. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Thyroglobulin present in the patient samples is determined directly from this curve.
### Test procedure

Prior to use in the assay allow all reagents and samples to come to room temperature (18-26 °C) and mix well. **Perform analysis always in duplicate**

Mark the positions of STD/SAMPLE/CTRL (Standard/Sample/Control) on a protocol sheet

Take microtiter strips out of the kit. Store unused strips covered at 2-8°C. Strips are stable until the expiry date stated on the label

Wash each well 5 times by dispensing 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper

Add 100 µl of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well

Cover the plate tightly and incubate for 3 hours at 37°C shaking on a horizontal mixer or 4 hours at 37°C without shaking. Alternatively, incubate **overnight at room temperature** on a horizontal mixer.

Discard the contents of each well. Wash 5 times by dispensing 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper
Add 100 µl CONJ (conjugate) into each well

Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) on a horizontal mixer

Discard the contents of each well. Wash 5 times by dispensing 250 µl of diluted WASHBUF (wash buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper

Add 100 µl of SUB (substrate) into each well

Incubate for 10 - 20 minutes at room temperature (18-26°C) in the dark*

Add 50 µl of STOP (stop solution) into each well, mix thoroughly

Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

*The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.
NOTE: The assay can be run automatically, e.g. using the ETILAB instrument of Sorin/Biomedica.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-Parameter-algorithm
   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e.g. 0.01).

2. Point-to-point-calculation
   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline-algorithm
   We recommend for the optical density a linear ordinate and for the concentration a logarithmic abscissa. When using a logarithmic abscissa, the zero calibrator has to be specified with a value smaller than 1 (e.g. 0.01).

THE CALIBRATION CURVE IS NOT LINEAR, therefore a point-to-point-calculation algorithm is recommended.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Serum and plasma samples
For the calculation of the thyroglobulin concentration in diluted plasma and serum samples the result has to be multiplied by 10.
9. **QUALITY CONTROL**

ALPCO Diagnostics recommends the use of commercial control samples for internal quality control if available.

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

*Expected values*

*Normal ranges*

Serum/plasma: $< 50 \mu g/l$

We recommend each laboratory to establish its own normal concentration range.

10. **PERFORMANCE CHARACTERISTICS**

*Sensitivity*

The detection limit of this hTG ELISA was determined to $B_0 + 2SD$. The limit is $1 \mu g/l$.

*Cross reactivity*

The described hTG assay is highly specific. No cross reactivity with other proteins in plasma and serum was observed.

*Linearity*

The linearity of the assay was determined by serial dilutions of material with known hTG concentration with the assay buffer. The linearity is in the range of $2.5 - 250 \mu g/l$.

*Precision data*

Intra- (CV%) and inter-assay (CV%) variations were determined using hTG containing samples within different concentration ranges. The intra-assay CV was 10.2 %, whereas 14.8 % was obtained for the inter-assay CV using three different kit batches.
11. PRECAUTIONS

- For research use only.
- The quality control guidelines should be followed.
- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Reagents of the kit package contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

12. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.
13. **GENERAL NOTES ON THE TEST AND TEST PROCEDURE**

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for research use only.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, not in accordance with manufacturer's recommendations, may influence the results of the test.