Thymosin (Alpha-1) EIA

For the quantitative determination of Thymosin alpha 1 in serum and thymus extracts.

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

Catalog Number: 30-9510
Size: 96 wells
Version: 18.12.2007 – ALPCO 12/1/08
1. INTENDED USE
This Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative
determination of Thymosin alpha 1 in serum and thymus extracts. This kit is for research
use only; it is not for use in diagnostic procedures.

2. SUMMARY AND EXPLANATION OF THE TEST
Thymosin alpha 1 was the first single peptide isolated from thymus fraction 5. It acts on T-
helper and NK-cells. Thymosin alpha 1 has been reported to exert effects on hormones
regulating the hypothalamus (1). Thymosin alpha 1 has been demonstrated to have
beneficial effects in animal models of liver and colon carcinoma (2) or leukemia (3). Its
use as a prognostic factor in human studies, e.g., colon carcinoma (4), has been
discussed. Thymosin alpha 1 has been successfully used as a component in combined
chemotherapy in bronchial carcinoma (5).

Areas of Research
- Disorders of the immune system
- Control of immune status in association with chemotherapy
- Disorder of endocrinium
- Quality control of thymus extracts

3. PRINCIPLE OF THE TEST
In this enzyme immunoassay (EIA) for the determination of Thymosin alpha 1 (Tα1)
polyclonal rabbit antibodies directed against synthetic Tα1 are used. The test principle is
based on competition between antigen in the sample or standards and the antigen coated
on the wells of microplate. A peroxidase-conjugated antibody is used for the detection and
quantification, and tetramethylbenzidine (TMB) is the peroxidase substrate. The enzymatic
reaction is terminated by an acidic stop solution. A dose response curve of absorbance unit
(optical density, OD at 450 nm) vs. concentration is generated using the values obtained
from the standards. The quantity of Tα1 present in the samples is determined directly from
this curve.
4. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Content</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 9510MTP</td>
<td>PLATE</td>
<td>One holder with precoated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K 9510WP</td>
<td>WASHBUF</td>
<td>ELISA wash buffer concentrate 10x</td>
<td>100 ml</td>
</tr>
<tr>
<td>K 9510A</td>
<td>AB</td>
<td>Antibody (rabbit anti-Thymosin α1), ready-to-use</td>
<td>3 x 3.5 ml</td>
</tr>
<tr>
<td>K 9510K</td>
<td>CONJ</td>
<td>Conjugate (goat anti rabbit, Peroxidase-labeled), ready-to-use</td>
<td>22 ml</td>
</tr>
<tr>
<td>K 9510VP</td>
<td>STDBUF</td>
<td>Standard dilution buffer, ready-to-use</td>
<td>50 ml</td>
</tr>
<tr>
<td>K 9510ST</td>
<td>STD</td>
<td>Calibrator concentrate, lyophilized</td>
<td>3 x 1 vials</td>
</tr>
<tr>
<td>K 9510TMB</td>
<td>SUB</td>
<td>TMB substrate (Tetramethylbenzidine), ready-to-use</td>
<td>2 x 15 ml</td>
</tr>
<tr>
<td>K 9510AC</td>
<td>STOP</td>
<td>ELISA stop solution, ready-to-use</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

5. MATERIALS REQUIRED BUT NOT SUPPLIED

- Deionized or distilled water.
- Precision pipettors calibrated and tips to deliver 5-1000 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 or 405 nm (reference wave length 620 or 690 nm)
6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each 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 WASHBUF (ELISA wash buffer concentrate) should be diluted with deionized water 1:10 before use (100 ml concentrate + 900 ml deionized water), mix well. Crystals could occur due to the high salt concentration in the stock solutions. The crystals must be dissolved at room temperature or at 37°C before dilution of the buffer concentrate. The buffer concentrate is stable at 2-8°C until the expiry date stated on the label. Diluted, working strength buffer solution can be stored in a closed flask at 2-8°C for one month.

- The AB (antibody) is ready to use. It can be stored at 2-8°C up to 4 weeks. Long time storage until the expiry date given on the label has to be at -20°C.

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

- The STD (standard) is delivered as a 1000 ng/ml concentrate. It must be reconstituted with 1 ml STDBUF (standard dilution buffer) and serially diluted 1:4 in steps according to the following scheme:

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Volume of Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>200 µl S1 + 600 µl buffer</td>
</tr>
<tr>
<td>S2</td>
<td>200 µl S2 + 600 µl buffer</td>
</tr>
<tr>
<td>S3</td>
<td>200 µl S3 + 600 µl buffer</td>
</tr>
<tr>
<td>S4</td>
<td>200 µl S4 + 600 µl buffer</td>
</tr>
</tbody>
</table>

The STDBUF (standard dilution buffer) is used as the Zero calibrator.

7. PRECAUTIONS

- Human materials used in kit components were 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.

- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrate for the enzymatic color reaction is 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 spills should be wiped up immediately with copious quantities of water.

• Reagents should not be used beyond the expiration date shown on the kit label.

8. SPECIMEN COLLECTION AND PREPARATION

Serum
Serum can be used without dilution. Store samples at -20 °C.

Thymus extract
Thymus extracts have varying compositions. Please contact the supplier when using thymus extracts.

9. ASSAY PROCEDURE

Procedural notes
• Do not interchange different lot numbers of any kit component within the same assay.
• Substrate solution should remain colorless until use.
• Proper adhesion of plate sealers during the incubation steps is necessary to ensure accurate results.
• Avoid foaming when mixing reagents.
• The assay should always be performed according to the enclosed manual.

Preincubation
1. Add 200 µl of the AB (antibody) to test tubes containing 400 µl of STD (calibrator) or sample solutions.
2. Incubate for 18 hours at 2-8°C on a shaker.

The volume is sufficient to assay the sample in duplicate.

Test procedure
Wash the precoated microtiter plate 5 x with 250 µl working strength ELISA wash buffer. Carry out the tests in duplicate.
1. Add 200 µl of the preincubation mixture to each well.
2. Incubate for 90 min at 2-8°C on a shaker in the dark.
3. Aspirate and wash the wells 5 x with 250 µl working strength ELISA wash buffer.
5. Incubate for **1 hour** at room temperature.

6. Aspirate and wash the wells **5 x with 250 µl** working strength ELISA wash buffer.

7. Add **200 µl** of **SUB** (TMB substrate).

8. Incubate for **20-30 minutes** at room temperature.

9. Add **50 µl** of **STOP** (stop solution) and mix briefly.

10. Immediately determine absorption with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference.

### 10. RESULTS

A calibration curve is constructed from the calibrators. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve.

THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL-algorithm is recommended.

**Typical calibration curve**

<table>
<thead>
<tr>
<th>Concentration [ng/ml]</th>
<th>1000</th>
<th>250</th>
<th>62</th>
<th>16</th>
<th>4</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD mean value</td>
<td>0.283</td>
<td>0.422</td>
<td>0.692</td>
<td>0.845</td>
<td>1.054</td>
<td>1.117</td>
</tr>
</tbody>
</table>

The data is for demonstration only and cannot be used for the evaluation of test results.
Thymus extract

In order to calculate the sample values the results from the microplate reader need to be multiplied by the selected dilution factor.

11. LIMITATIONS

Samples with levels greater than the highest standard value should be diluted and re-assayed.

12. QUALITY CONTROL

The use of commercial control samples is recommended for internal quality control.

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

We recommend each laboratory establishes its own baseline values.

Baseline values depend on the individual’s age and vary between different individuals.

13. REFERENCES

1. Melatonin is responsible for the nocturnal increase observed in serum and thymus of thymosin alpha 1 and thymulin concentrations: observation in rats and humans. Molinero P et al. (2000) J Neuroimmunol 103:180-188


3. Anti-Tumor Effect of Combined Treatment with Thymosin alpha 1 and Interleukin-2 after 5-Fluorouracil in Liver Metastases from Colorectal Cancer in Rats. Rasi et al. (1994) Int J Cancer 57:701-705

4. Antitumor Effect of Thymosin α1/Interleukin-2 or Thymosin α1/Interferon α, β Following Cyclophosphamide in Mice Injected with Highly Metastatic Friend Erythroleukemia Cells. Garaci et al. (1 993) J Immunotherapy 13:7-17


14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.

- All reagents in the kit package are for RESEARCH use only.

- Guidelines for medical laboratories should be observed.

- Incubation time, incubation temperature, and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. ALPCO can therefore not be held responsible for any damage resulting from wrong use.

- Warranty claims and complaints in respect to deficiencies must be logged within 14 days after receipt of the product. The product shall be sent to ALPCO along with a written complaint.