Intended Use

The PromoKine Thymosin α1 ELISA is intended for the quantitative determination of Thymosin β4 in serum and thymus extract. It is for research use only.

Summary and Explanation of the Test

Thymosin α1 was the first single peptide isolated from thymus fraction 5. It acts on T-helper and NK-cells. Thymosin α1 has been reported for exert effects on hormon regulating the hypothalamus (1). Thymosin α1 has been demonstrated to have beneficial effects in animal models of liver and colon carcinoma (2) or leukaemia (3). Its use as a prognostic factor in human studies, e.g. colon carcinoma (4) has been discussed. Thymosin α1 has been successfully used as component combined chemotherapy in bronchial carcinoma (5).

Indications

- Disorder of immune system
- Control of immune status in association with a chemotherapy
- Disorder of endocrinium
- Quality control of thymus extracts

Principle of the Test

In this enzyme immunoassay (EIA) for the determination of Thymosin α1 (T α1) polyclonal rabbit antibodies directed against synthetic T α1 are used. The test principle is based on a competition between antigen in the sample or standards and the antigen coated on the wells of microplate. A peroxidase-conjugated antibody is used for detection and quantification, and tetramethylbenzidine (TMB) as a 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 standard. T α1 present in the patient samples is determined directly from this curve.
Kit Components

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>One holder with precoated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>ELISA wash buffer concentrate 10x</td>
<td>100 ml</td>
</tr>
<tr>
<td>Antibody (rabbit anti Thymosin ( \alpha )) ready-to-use</td>
<td>3 x 3.5 ml</td>
</tr>
<tr>
<td>Conjugate (goat anti rabbit, Peroxidase-labeled), ready-to-use</td>
<td>22 ml</td>
</tr>
<tr>
<td>Dilution buffer, ready-to-use</td>
<td>50 ml</td>
</tr>
<tr>
<td>Calibrator concentrate, lyophilized</td>
<td>3 x 1 vials</td>
</tr>
<tr>
<td>TMB substrate (Tetramethylbenzidine), ready-to-use</td>
<td>2 x 15 ml</td>
</tr>
<tr>
<td>ELISA stop solution, ready-to-use</td>
<td>7 ml</td>
</tr>
</tbody>
</table>

Material Required but not Supplied

- Bidistilled water (aqua bidest.)
- 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)

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 **ELISA wash buffer concentrate** (WASHBUF) should be diluted with aqua bidest. 1:10 before use (100 ml concentrate + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redisolved at room temperature or at 37°C before dilution of the buffer solutions. 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 **antibody** is ready to use. It could 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 **standard** is delivered as 1000 ng/ml concentrate, must be reconstituted with 1 ml dilution

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>(1000 ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 μl S1</td>
<td>+ 600 μl buffer = S2</td>
<td>(250 ng/ml)</td>
</tr>
<tr>
<td>200 μl S2</td>
<td>+ 600 μl buffer = S3</td>
<td>(62 ng/ml)</td>
</tr>
<tr>
<td>200 μl S3</td>
<td>+ 600 μl buffer = S4</td>
<td>(16 ng/ml)</td>
</tr>
<tr>
<td>200 μl S4</td>
<td>+ 600 μl buffer = S5</td>
<td>(4 ng/ml)</td>
</tr>
</tbody>
</table>

Dilution buffer is used as a Zero calibrator.
Warnings and Precautions for Users

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. 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 spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on the kit label.

Sample Collection and Storage

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.

Assay Procedure

Procedural notes
- Do not interchange different lot numbers of any kit component within the same assay.
- 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 to the enclosed manual.

Preincubation
- Add 200 μl of the 1st antibody to 400 μl of calibrator or sample solutions into one way test tubes.
- 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 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 ELISA wash buffer
4. Add 200 μl of the anti-rabbit-POD-conjugate
5. Incubate for 1 hour at room temperature
6. Aspirate and wash the wells 5 x with 250 μl ELISA wash buffer
7. Add 200 μl TMB substrate
8. Incubate for 20-30 minutes at room temperature
9. Add 50 μl stop solution and mix briefly
10. Immediately determine absorption with an ELISA reader at 450 nm against 620 nm (or 690 nm) as reference

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

![Typical calibration curve graph](image)

<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>0.261</td>
<td>0.444</td>
<td>0.638</td>
<td>0.865</td>
<td>1.073</td>
<td>1.266</td>
</tr>
</tbody>
</table>

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

**Thymus extract**

For the calculation of the sample values the results from the microplate reader has to be multiplied with the selected dilution factor.

**Limitations**

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

**Quality Control**

PromoCell recommends commercially control samples as 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 patient 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 to establish its own baseline values. Baseline values depend on the patient’s age and vary between different individuals.

**References**

- Melatonin is responsible for the nocturnal increase observed in serum and thymus of thymosin alpha1 and thymulin concentrations: observation in rats and humans. **Molinero P et al.** (2000) *J Neuroimmunol* 103:180-188
- 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
- Determination of Thymosin α1 with enzyme-immunoassay in colorectal cancer patients. Jevromovic et al. (1997) Archive of Oncology 5:193

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 *in vitro* diagnostic 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. PromoCell can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to PromoCell along with a written complaint.

### Ordering Information

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Product Description</th>
<th>Size</th>
<th>Catalog Number</th>
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<tbody>
<tr>
<td>Thymosin α1 ELISA Kit, human</td>
<td>Human Thymosin alpha-1 ELISA Kit</td>
<td>96 Tests</td>
<td>PK-EL-K9510</td>
</tr>
</tbody>
</table>

*For in vitro research use only. Not for diagnostic or therapeutic procedures.*

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