Thymosin α1 ELISA

For the determination of thymosin α1 in serum and thymus extract

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1. INTENDED USE
This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of thymosin α1 in serum and thymus extract. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION
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 hormone 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).

Possible research areas
- Disorder of immune system
- Control of immune status in association with a chemotherapy
- Disorder of endocrinum
- Quality control of thymus extracts

3. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Label</th>
<th>Kit components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KR9510</td>
<td>PLATE</td>
<td>Microtiter plate, pre-coated</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>KR0001.C.100</td>
<td>WASHBUF</td>
<td>Wash buffer concentrate, 10 x</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>KR9510</td>
<td>CONJ</td>
<td>Conjugate (goat anti rabbit, peroxidase-labelled), ready-to-use</td>
<td>1 x 22 ml</td>
</tr>
<tr>
<td>KR9510</td>
<td>STD</td>
<td>Standard concentrate, lyophilised (see specification for concentration)</td>
<td>4 x 1 vial</td>
</tr>
<tr>
<td>KR9510</td>
<td>AB</td>
<td>Antibody (rabbit anti thymosin α1), ready-to-use</td>
<td>3 x 3.5 ml</td>
</tr>
<tr>
<td>KR9510</td>
<td>STDBUF</td>
<td>Standard dilution buffer, ready-to-use</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>KR0002.15</td>
<td>SUB</td>
<td>Substrate (tetramethylbenzidine), ready-to-use</td>
<td>2 x 15 ml</td>
</tr>
<tr>
<td>KR0003.15</td>
<td>STOP</td>
<td>Stop solution, ready-to-use</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>

For reorders of single components, use the catalogue number followed by the label as product number.
4. **MATERIAL REQUIRED BUT NOT SUPPLIED**

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25°C (≥ 18.2 MΩ•cm).

5. **STORAGE AND PREPARATION OF REAGENTS**

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 3 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.

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

- The **antibody** (AB) 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.

- The **lyophilised standard concentrate** (STD) is stable at **2–8°C** until the expiry date stated on the label. **Reconstitution details, preparation of the standard curve** as well as **concentrations and ranges** are given in the specification data sheet. Standards S1–S5 are not stable and cannot be stored.

- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8°C**.
6. STORAGE AND PREPARATION OF SAMPLES

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

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

7. ASSAY PROCEDURE

Principle of the test
This ELISA is designed for the quantitative determination of thymosin α1 (Tα1).
In this test, polyclonal rabbit antibodies directed against synthetic Tα1 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. Tα1, present in the samples, is determined directly from this curve.

Test procedure
Bring all reagents and samples to room temperature (15–30 °C) and mix well.
Mark the positions of standards and samples on a protocol sheet.
Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until the expiry date stated on the label.
For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.
We recommend to carry out the tests in duplicate.

Preincubation

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1.</td>
<td>Pipet each <strong>400 μl standard</strong> (S1–S6) or <strong>sample</strong> into a test tube.</td>
</tr>
<tr>
<td>2.</td>
<td>Add <strong>200 μl antibody</strong> (AB) into each test tube.</td>
</tr>
</tbody>
</table>
3. Incubate for **18 hours at 2–8 °C on a horizontal shaker***.

The given volumes are for tests in duplicate.

**Incubation on the microtiter plate (PLATE)**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.</td>
<td>Discard the content of each well and wash <strong>5 times</strong> with <strong>250 µl wash buffer</strong>. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.</td>
</tr>
<tr>
<td>5.</td>
<td>Add <strong>200 µl</strong> of the <strong>preincubated mixture</strong> into the respective wells.</td>
</tr>
<tr>
<td>6.</td>
<td>Cover the strips and incubate for <strong>90 min at 2–8 °C in the dark on a horizontal shaker</strong>*.</td>
</tr>
<tr>
<td>7.</td>
<td>Discard the content of each well and wash <strong>5 times</strong> with <strong>250 µl wash buffer</strong>. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.</td>
</tr>
<tr>
<td>8.</td>
<td>Add <strong>200 µl conjugate</strong> (CONJ) in each well.</td>
</tr>
<tr>
<td>9.</td>
<td>Cover the strips and incubate for <strong>1 hour</strong> at room temperature (15–30 °C) <strong>on a horizontal shaker</strong>*.</td>
</tr>
<tr>
<td>10.</td>
<td>Discard the content of each well and wash <strong>5 times</strong> with <strong>250 µl wash buffer</strong>. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.</td>
</tr>
<tr>
<td>11.</td>
<td>Add <strong>200 µl substrate</strong> (SUB) in each well.</td>
</tr>
<tr>
<td>12.</td>
<td>Incubate for <strong>10–20 minutes</strong>** at room temperature (15–30 °C) in the dark.</td>
</tr>
<tr>
<td>13.</td>
<td>Add <strong>50 µl stop solution</strong> (STOP) and mix well.</td>
</tr>
<tr>
<td>14.</td>
<td>Determine <strong>absorption immediately</strong> with an ELISA reader at <strong>450 nm</strong> 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 <strong>405 nm</strong> against 620 nm as a reference.</td>
</tr>
</tbody>
</table>

*** We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.
8. RESULTS
The following algorithms can be used alternatively to calculate the results. We recommend using 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 standard must be specified with a value less than 1 (e.g. 0.001).

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 a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

**Thymus extract**
The obtained results have to be multiplied with the selected dilution factor to get the actual concentrations.

9. LIMITATIONS
Samples with an OD lower than the OD of the highest standard should be further diluted and re-assayed. For the following analysis, the changed dilution factor has to be taken into consideration.

10. QUALITY CONTROL
Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed 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.
Reference range
The values in healthy people are strongly age-dependent. Newborns show the highest values, and from the age of 20 onwards the serum values are falling. We recommend each laboratory to establish its own reference range for different age ranges.

11. PRECAUTIONS

- All reagents in the kit package are for research use only.
- 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 ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still should be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

12. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated 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.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.
13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for 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, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

14. REFERENCES

1. 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


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


Used symbols:

- Temperature limitation
- Catalogue Number
- For research use only
- To be used with
- Manufacturer
- Contains sufficient for \(<\text{n}\) tests
- Lot number
- Use by
- Attention
- Consult instructions for use
- Consult specification data sheet