Thymulin EIA Kit
Zur in vitro Bestimmung von Thymulin in Serum und Thymusextrakt

Thymulin EIA Kit
For the in vitro determination of thymulin in serum and thymus extract

Nur zu wissenschaftlichen Zwecken / For research use only

Gültig ab / Valid from 30.01.2008
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1. INTENDED USE

The Immundiagnostik Assay is intended for the quantitative determination of Thymulin in serum and thymus preparations. It is for research use only.

2. SUMMARY AND EXPLANATION OF THE TEST

The thymus regulates multiple functions. It is mainly responsible for the immune reactions. In addition, it influences the central nervous system and the endocrinium by the secretion of single peptides like Thymulin and Thymosines. Thymulin is only active as a zinc complex, and acts on T-lymphocytes and their precursor stem cells. The secretion of Thymulin is regulated by the pituitary gland.

Indication

- Immune dysfunction and immune deficiencies, e.g. leukaemia, AIDS
- Autoimmune disease, e.g. systemic Lupus erythematoses, rheumatoid arthritis, multiple sclerosis
- Zinc dependent diseases, e.g. Morbus Crohn
- Dysfunction of the endocrinium
- Quality control of thymus preparations

3. PRINCIPLE OF THE TEST

This enzyme immuno assay (EIA) can be used for the determination of Thymulin in serum and thymus preparations.

The test principle is based on a competition between the antigen in the sample or standards and biotinylated thymulin as a tracer for the binding sites of anti-Thymulin antibodies coated on the wells of the microplate. A peroxidase-conjugated steptavidin 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 the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standards. Thymulin present in the patient samples is determined directly from this curve.
4. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Cat. No</th>
<th>Label</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 9810MTP</td>
<td>PLATE</td>
<td>Microtiter plate, precoated</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K 9810WP</td>
<td>WASHBUF</td>
<td>ELISA wash buffer concentrate 10x</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>K 9810AP</td>
<td>ASYBUF</td>
<td>Assay buffer</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>K 9810T</td>
<td>TRACER</td>
<td>Tracer (Biotinylated Thymulin), ready to use</td>
<td>3 x 2 ml</td>
</tr>
<tr>
<td>K 9810K</td>
<td>CONJ</td>
<td>Conjugate (streptavidin-peroxidase-labeled), ready to use</td>
<td>22 ml</td>
</tr>
<tr>
<td>K 9810ST</td>
<td>STD</td>
<td>Calibrators, lyophilized (0; 0.03; 0.13; 0.64; 3.2; 16 ng/ml)</td>
<td>3 x 6 vials</td>
</tr>
<tr>
<td>K 9810KO</td>
<td>CTRL</td>
<td>Control, lyophilized</td>
<td>3 x 1 vial</td>
</tr>
<tr>
<td>K 9810TMB</td>
<td>SUB</td>
<td>TMB substrate (Tetramethylbenzidine)</td>
<td>2 x 15 ml</td>
</tr>
<tr>
<td>K 9810AC</td>
<td>STOP</td>
<td>ELISA stop solution, ready to use</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>

5. 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)
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 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.

- The **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution of the buffer solutions. The WASHBUF 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 **TRACER** (tracer, biotinylated Thymulin) must be stored at **-20°C** and is stable until the expiry date stated on the label.

- The lyophilized **STD** (standards) and **CTRL** (control) must be reconstituted with **150 µl** aqua bidest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. **Reconstituted standards and control are not stable and can not be stored.**

- 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**.

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. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
• Stop solution is composed of sulphuric 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.

8. SPECIMEN COLLECTION AND PREPARATION

**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.

9. 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 the enclosed manual.
Test procedure

Wash the PLATE (precoated microtiter plate) 5 x with 250 µl diluted wash buffer. Carry out the tests in duplicate.

1. Pipette 50 µl of STD (standards), CTRL (control) or samples and 150 µl of the ASYBUF (assay buffer) into each well.
2. Incubate for 1 hour at room temperature shaking on a horizontal mixer.
3. Add 50 µl of the TRACER (tracer, biotinylated thymulin) into each well, shake gently.
4. Incubate for 16 - 20 hours at 2-8°C shaking on a horizontal mixer.
5. Decant the contents of the plate and wash the wells 5 x with 250 µl of diluted wash buffer.
6. Add 200 µl of CONJ (conjugate) solution into each well.
7. Incubate for 1 hour at room temperature shaking on a horizontal mixer.
8. Decant the contents of the plate and wash the wells 5 x with 250 µl of diluted wash buffer.
9. Add 200 µl of SUB (TMB-substrate) solution
10. Incubate for 15 ± 5 min at room temperature in the dark until sufficient coloring is achieved.
11. Add 50 µl of STOP (stop solution) and mix shortly.
12. 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 measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm (or 690 nm) as 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.

11. LIMITATIONS

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

12. QUALITY CONTROL

Immundiagnostik 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

Reference range

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

We recommend each laboratory to establish its own baseline values.
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. 

2. Effect of Thymosin α1 on Hypothalamic Hormone Release. 
   Milenkovic et al. (1992): Neuroendocrinol. 56: 674-679

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. (1993) J Immunotherapy 13:7-17

5. Determination of Thymosin α1 with enzyme-immunoassay in colorectal cancer patients. 
   Jevromovic et al. (1997) Archive of Oncology 5:193

6. A randomized trial to evaluate the immunorestorative propertise of synthetic Thymosin α1 in patients with lung cancer. 
   Schulof et al. (1985) Journ Biol resp Med 4,147-158

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 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. Immundiagnostik AG 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 Immundiagnostik AG along with a written complaint.
Used symbols:

- **Temperature limitation**
- **Catalogue Number**
- **In Vitro Diagnostic Medical Device**
- **Contains sufficient for \(<n\) tests**
- **Manufacturer**
- **Use by**
- **Lot number**