Arbeitsanleitung / Manual

Thymulin ELISA

Zur Bestimmung von Thymulin in Serum und Thymusextrakt

For the determination of thymulin in serum and thymus extract

Gültig ab / Valid from 2020-01-28 REV001

REF **KR9810**

Σ 96

+8 °C / +2 °C

RUO

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1. INTENDED USE

This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of thymulin in serum and thymus preparations.

For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

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 alpha 1 and beta 4. 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.

Possible research areas

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

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR9810	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	ELISA wash buffer concentrate, 10 x	1 x 100 ml
KR9810	CONJ	Conjugate (streptavidin-peroxidase-labeled), ready-to-use	1 x 22 ml
KR9810	STD	Standards, lyophilised (0; 0.03; 0.13; 0.64; 3.2; 16 ng/ml)	3 x 6 vials
KR9810	CTRL	Control, lyophilised (see specification for range)	3 x 1 vial
KR9810	TRACER	Tracer (biotinylated thymulin), lyophilised	1 x 1 vial
KR9810	ASYBUF	Assay buffer, ready to use	1 x 100 ml
K 0002.15	SUB	TMB substrate (tetramethylbenzidine), ready-to-use	2 x 15 ml
KR9810	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

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4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipettors and 5–1 000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3 000 *g*
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of Ultra Pure 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 ultra pure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solution. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. 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 one month**.
- The **lyophilised TRACER** (tracer, biotinylated thymulin) is stable at **2–8 °C** until the expiry date stated on the label. Before use, the lyophilised TRACER has to be reconstituted with **6 ml of ultra pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly to ensure complete reconstitution. **Reconstituted tracer** can be stored at –20 °C. The reconstituted tracer is stable at –20 °C until the expiry date stated on the label.
- The **lyophilised standards** (STD) and **control** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. Before use, the standards and controls have to be reconstituted with **150 µl of ultra pure water**. Allow the vial con-

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tent to dissolve for 10 minutes and mix thoroughly to ensure complete reconstitution. **Reconstituted standards and control 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 of test package) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Serum

For testing in duplicates, pipette **2 x 50 µl** of each sample per well.

Serum sample storage

Serum can be stored at -20 °C.

Thymus extract

Thymus extracts have varying compositions. For details please contact your supplier or Immundiagnostik AG.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative 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 streptavidin 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.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples (reconstituted STDs/reconstituted CTRLs/SAMPLEs) on a protocol sheet.

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Take as many microtiter strips as needed from kit. Store unused strips in the aluminium packaging at 2–8 ° C. Strips are stable until 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.

1.	Wash the pre-coated microtiter plate (PLATE) **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
2.	Add **150 µl assaybuffer** (ASYBUF) into each well.
3.	Add **50 µl standards/controls/samples** into the respective wells.
4.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) **shaking** on a horizontal mixer.
5.	Add **50 µl tracer** (reconstituted TRACER) into each well, shake gently.
6.	Cover the strips and incubate for **16–20 hours** at 2–8 °C **shaking** on a horizontal mixer.
7.	Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8.	Add **200 µl conjugate** (CONJ) in each well.
9.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) **shaking** on a horizontal mixer.
10.	Discard the contents of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
11.	Add **200 µl TMB substrate** (SUB) in each well.
12.	Incubate for **10–20 minutes*** at room temperature (15–30 °C) in the dark.
13.	Add **50 µl ELISA stop solution** (STOP) and mix well.

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14.	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 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.

In case **a dilution factor** has been used, multiply the obtained result with the dilution factor used to get the real concentration.

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.

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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 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.

Reference range

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

We recommend each laboratory to establish its own reference range.

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 must 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 kit label.
- Substrate solution should remain colourless until use.

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- 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 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. Garaci, E. et al., 1993. Antitumor effect of thymosin alpha 1/interleukin-2 or thymosin alpha 1/interferon alpha,beta following cyclophosphamide in mice injected with highly metastatic Friend erythroleukemia cells. *Journal of immunotherapy with emphasis on tumor immunology : official journal of the Society for Biological Therapy*, **13**(1), pp.7–17.
2. Jevremovic, M. et al., 1997. Determination of thymosin alpha1 with enzyme-immunoassay in colorectal cancer patients. *Archive of Oncology*, **5**(4), pp.193–194.
3. Milenkovic, L. et al., 1992. Effect of thymosin alpha 1 on hypothalamic hormone release. *Neuroendocrinology*, **56**(5), pp.674–679.
4. Molinero, P. et al., 2000. Melatonin is responsible for the nocturnal increase observed in serum and thymus of thymosin alpha1 and thymulin concentrations: observations in rats and humans. *Journal of neuroimmunology,* **103**(2), pp.180–8.
5. Rasi, G. et al., 1994. Anti-tumor effect of combined treatment with thymosin alpha 1 and interleukin-2 after 5-fluorouracil in liver metastases from colorectal cancer in rats. *International journal of cancer. Journal international du cancer*, **57**(5), pp.701–5.

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6. Schulof, R.S. et al., 1985. A randomized trial to evaluate the immunorestorative properties of synthetic thymosin-alpha 1 in patients with lung cancer. *Journal of biological response modifiers*, **4**(2), pp.147–58.

Used symbols:


Temperature limitation

Catalogue number

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To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet

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