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Manual

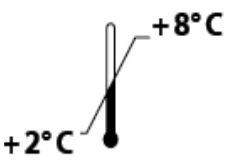
Tyrosine ELISA

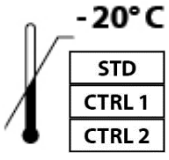
For the in vitro determination of L-tyrosine in human EDTA plasma and serum

For research use only

Valid from 2019-02-05

REF **KR7015**





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1. INTENDED USE

This Immundiagnostik AG assay is intended for the quantitative determination of L-tyrosine in human EDTA plasma and serum. For research use only. Not for use in diagnostic procedures.

2. MATERIAL SUPPLIED

Cat. No	Label	Kit Components	Quantity
KR7015	PLATE	Holder with precoated strips	12 x 8 wells
KR7015	STD	Standards, ready-to-use (0, 6, 20, 60, 200, 600 µmol/l)	6 x 200 µl
KR7015	CTRL 1	Control, ready-to-use (see specification for range)	1 x 200 µl
KR7015	CTRL 2	Control, ready-to-use (see specification for range)	1 x 200 µl
KR0006.C.100	WASHBUF A	Wash buffer concentrate, 10 x	2 x 100 ml
KR7015	AB	L-tyrosine antibody, lyophilised	2 x 1 vial
KR7015	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 65 µl
KR0010.13	CONJBUF	Conjugate stabilizing buffer, ready-to-use	1 x 13 ml
KR7015	REABUF	Reaction buffer, ready-to-use	1 x 100 ml
KR7015	DER	Derivatisation reagent, lyophilised	2 x 13.3 mg
KR0008.04	DMSO	Dimethylsulfoxide (DMSO)	1 x 4 ml
KR0011.28	ASYBUF	Assay buffer, ready-to-use	2 x 28 ml
KR7015	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR7015	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.

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipets and 10-1000 µl tips

- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Centrifuge, 3000 *g*
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 6)

* 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).

4. 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 assay**. The kit can be used up to 2 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 A)** has to be diluted with ultra pure water **1:10** before use (100 ml WASHBUF A + 900 ml ultra pure 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 A** is stable at **2-8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF A) can be stored in a closed flask at **2-8 °C for 1 month**.

- Store **standards and controls (STD/CTRL)** frozen at **-20 °C**. They are stable at -20 °C until the expiry date stated on the label. Thaw before use in the test and mix well. Re-freeze standards and controls after use.

- **DMSO** crystallises at 2-8 °C. Before use, bring to room temperature to dissolve the crystals.

- Reconstitute the content of one vial of **derivatisation reagent (DER)** (13.3 mg) **with 0.8 ml DMSO.** Allow to dissolve for 10 minutes and mix thoroughly with a vortex-mixer. The derivatisation reagent must be **prepared immediately before use**. When more than one vial is to be used, combine the contents and mix prior to use. Discard any rest of the reagent after use. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

- The **lyophilised L-tyrosine antibody (AB)** is stable at **2-8 °C** until the expiry date stated on the label. Dissolve the content of one vial of AB in **5.5 ml of wash buffer**. When more than one vial is to be used, combine the contents and mix prior to use. **L-tyrosine antibody** (reconstituted AB) **can be stored at -20 °C for 1 month.**
- **Preparation of the conjugate**: Before use, the conjugate concentrate has to be diluted **1:201** with **conjugate stabilizing buffer (CONJBUF)** (e.g. 60 µl CONJ + 12 ml CONJBUF). The CONJ is stable at **2-8 °C** until the expiry date stated on the label. **Conjugate** (1:201 diluted CONJ) **can be stored at 2-8 °C for 1 month.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label) when stored at **2-8°C.**

5. STORAGE AND PREPARATION OF SAMPLES

Serum and EDTA plasma

- Serum and EDTA-Plasma samples are stable for up to 6 hours at 2-8°C. For longer storage, keep samples frozen at -20 °C.
- The EDTA plasma and serum samples are diluted for derivatisation (see sample preparation procedure).
- For sample preparation, a derivatisation reagent for derivatisation of L-tyrosine is added (see sample preparation procedure).

6. ASSAY PROCEDURE

Principle of the test

This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of a derivatisation reagent for L-tyrosine derivatisation. Afterwards, the treated samples and a polyclonal L-tyrosine antiserum are incubated in wells of a microtiter plate coated with L-tyrosine-derivative (tracer). During the incubation period, the target L-tyrosine in the sample competes with the tracer, immobilised on the wall of the microtiter wells, for the binding of the polyclonal antibodies. The L-tyrosine in the sample displaces the antibodies out of the binding to the tracer

During the second incubation step, a peroxidase conjugate is added to each microtiter well to detect the anti-L-tyrosine antibodies. After washing away the unbound components tetramethylbenzidine is added as peroxidase substrate.

Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow, and the absorbance is measured in a photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the L-tyrosine concentration in the sample; this means, high L-tyrosine concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal.

A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. L-tyrosine, present in the patient samples, is determined directly from this curve.

Sample preparation procedure

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

Dilute standards (STD), controls (CTRL) and samples **1:41** with reaction buffer as follows:

25 µl STD / CTRL / sample + 1000 µl REABUF (reaction buffer)

Please note: Samples from patients with L-tyrosine supplementation (e.g. in depletion studies) probably require further dilution, we recommend diluting these samples additionally 1:2 with REABUF.

Derivatisation of the diluted standards, controls and samples is carried out in single analysis in vials (e.g. 1.5 ml vials).

The reagents provided with this kit are sufficient for up to 48 derivatisations, which are transferred in duplicate determinations to the wells of the microtiter plate.

1.	Add **100 µl of diluted standards/controls/samples** into the corresponding vials.
2.	Add **25 µl** of freshly prepared **derivatisation reagent** into each vial (STD, CTRL, sample), **mix thoroughly** by repeated inversion or several seconds on a vortex mixer. Incubate for **45 min at room temperature** (15-30 °C) on a **horizontal shaker**.
3.	Add **1000 µl assay buffer** (ASYBUF) into each vial, mix well and incubate for **45 min at room temperature** (15-30°C) on a **horizontal shaker.**

2 x 25 µl of the derivatised standards, controls and samples are used in the ELISA as duplicates.

Test procedure

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered at 2-8 °C. Strips are stable until the expiry date stated on the label.

4.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by fimly tapping the plate on absorbent paper.
5.	For the analysis in duplicate, take **2 x 25 µl** of the **derivatised standards/ controls/samples** out of the vials and add into the respective wells of the microtiter plate.
6.	Add **100 µl L-tyrosine antibody** into each well of the microtiter plate.
7.	Cover the strips tightly with foil and incubate **overnight at 2-8°C**.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by fimly tapping the plate on absorbent paper.
9.	Add **100 µl conjugate** into each well.
10.	Cover the strips and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.
11.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by fimly tapping the plate on absorbent paper.
12.	Add **100 µl substrate** (SUB) into each well.
13.	Incubate for **8-12 min*** at room temperature (15-30 °C) in the **dark**.
14.	Add **100 µl stop solution** (STOP) into each well and mix well.
15.	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 (690 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.

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.

7. 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 optical density and a logarithmic abscissa for 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 optical density and a linear abscissa for concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

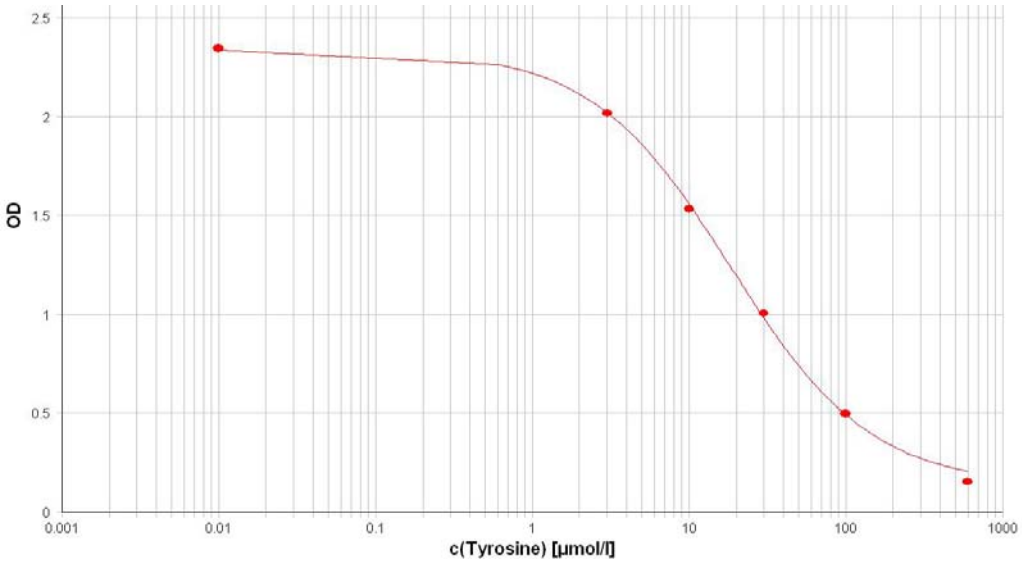
The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

EDTA plasma and serum

The concentrations can be determined directly from the standard curve in µmol/l. **No factor** is required.

The results from additionally diluted samples must be multiplied by this dilution factor.

In the following, an example of a calibration curve is given; do not use it for the calculation of your results.



8. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted with reaction buffer (READUF) and re-assayed. Please consider this dilution factor when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × sample dilution factor to be used

Analytical sensitivity see chapter "Performance Characteristics".

9. QUALITY CONTROL

Immundiagnostik AG 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 samples are outside of the acceptable limits.

Reference Range

Based on internal studies with plasma samples of apparently healthy persons (n=146) a mean value of 58 µmol/l was calculated. The standard deviation was

14.4 µmol/l. From mean value ± 2 x SD a normal range of 29 – 87 µmol/l was estimated.

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay (n=19)

sample	L-tyrosine [µmol/l]	CV [%]
1	31.4	7,6
2	125.3	7.4

Inter-assay (n=8)

sample	L-tyrosine [µmol/l]	CV [%]
1	69.7	5.8
2	104.6	4.6

Spiking recovery

Two plasma samples were spiked with different L-tyrosine concentrations and measured in this assay. The mean recovery rate for all concentrations was 93.2 % (n=5).

sample [µmol/l]	spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
76.8	25	101.8	94.5	92.8
76.8	50	126.8	123.3	97.2
108.8	25	133.8	116.1	86.8
108.8	50	158.8	152.4	96.0

Dilution recovery

Two spiked plasma sample were diluted and measured in this assay. The mean recovery was 97.1 % (n=5).

sample [µmol/l]	dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
76.8	1:2	38.4	34.0	88.4
76.8	1:4	19.2	20.0	103.9
108.8	1:2	54.4	50.6	93.0
108.8	1:4	27.2	28.0	102.9

Analytical sensitivity

The zero-standard was measured 82 times. The detection limit was set as B_0 - 2 SD and estimated to be 3.6 µmol/l.

Specificity

Specificity was tested by measuring the cross-reactivity against compounds with structural similarity to L-tyrosine. The specificity is calculated in percent in relation to the L-tyrosine binding activity:

L-phenylalanine	< 2 %
L-tryptophan	< 0.5 %

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 sulfuric 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 breathe 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 medical 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 sent to Immundiagnostik AG along with a written complaint.

14. REFERENCES

General literature

- Badawy AA, Dougherty DM, Richard DM. Specificity of the acute tryptophan and tyrosine plus phenylalanine depletion and loading tests I. Review of biochemical aspects and poor specificity of current amino Acid formulations. Int J Tryptophan Res. 2010 Jan 1;2010(3):23-34.
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Competing Amino Acid Ratios in a New Control Formulation. Int J Tryptophan Res. 2010;3:35-47.

Literature using Immundiagnostik AG Tyrosine ELISA [KR7015]

- Hildebrand P, Königschulte W, Gaber TJ, Bubenzer-Busch S, Helmbold K, Biskup CS, Langen KJ, Fink GR, Zepf FD: Effects of dietary tryptophan and phenylalanine-tyrosine depletion on phasic alertness in healthy adults - A pilot study. *Food & nutrition research*. 2015;**59**:26407. doi:10.3402/fnr.v59.26407.

Used symbols: