Manual

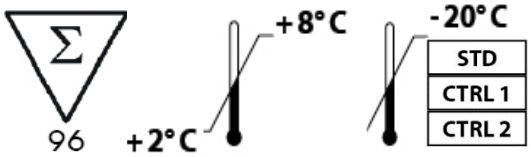
IDK® Arginine ELISA

For the in vitro determination of of L-arginine in EDTA plasma

For research use only

Valid from 2021-07-19

REF **KR7733**



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1. INTENDED USE

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

2. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR7733	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR7733	STD	Standards, ready-to-use (0, 12.5, 30, 60, 120, 300 µmol/l)	6 x 500 µl
KR7733	CTRL 1	Control, ready-to-use (see specification for range)	1 x 500 µl
KR7733	CTRL 2	Control, ready-to-use (see specification for range)	1 x 500 µl
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR7733	SAMPLEBUF	Sample buffer, ready-to-use	1 x 100 ml
KR0011.70	ASYBUF	Assay buffer, ready-to-use	2 x 70 ml
KR7733	AB	L-arginine antibody, lyophilised	2 x 1 vial
KR7733	CONJ	Conjugate, peroxidase-labelled, ready-to-use	1 x 12 ml
KR7733	DER	Derivatisation reagent, lyophilised	1 x 50 mg
KR0008.04	DMSO	Dimethylsulfoxide (DMSO)	1 x 4 ml
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	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

- 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.

- **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 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**.

- 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.

- The **lyophilised derivatisation reagent (DER)** is stable at **2-8 °C** until the expiry date stated on the label. Bring to room temperature before opening. Reconstitute the DER (50 mg) with **3 ml DMSO**. Allow to dissolve for 10 minutes and mix thoroughly with a vortex-mixer. **The derivatisation reagent** (reconstituted DER) **can be stored at 2-8 °C for 2 months**. Bring to room temperature before reuse. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

- The **lyophilised L-arginine 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 **9 ml of wash buffer**. When more than one vial is to be used, combine the contents and mix prior to use. **L-arginine antibody** (reconstituted AB) **can be stored at 2-8 °C for 2 months.**

- 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

EDTA plasma

- EDTA plasma is suited for this test system. For longer storage, keep samples frozen at -20 °C.
- The plasma samples are diluted before performing the assay (see derivatisation procedure).
- For sample preparation a derivatisation reagent for derivatisation of L-arginine is added (see sample derivatisation procedure).

6. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of L-arginine. The assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatisation reagent for L-arginine derivatisation. Afterwards, the treated samples and a polyclonal L-arginine antiserum are incubated in wells of a microtiter plate coated with L-arginine-derivative (tracer). During the incubation period, the target L-arginine in the sample competes with the tracer, immobilised on the wall of the microtiter wells, for the binding of the polyclonal antibodies.

During the second incubation step, a peroxidase-conjugated antibody is added to detect the anti-L-arginine antibodies. After washing away the unbound components, tetramethylbenzidine (TMB) is added as a 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-arginine concentration in the sample; this means, high L-arginine concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal. 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. L-arginine, present in the patient samples, is determined directly from this curve.

Derivatisation procedure

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

Dilute the EDTA plasma samples **1:41** with sample buffer as follows:

25 µl sample + **1 ml** sample buffer (SAMPLEBUF), mix well.

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

We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1.	Add **100 µl standard** (STD)/ **control** (CTRL)/ **diluted sample** into the corresponding vials.
2.	Add **25 µl derivatisation reagent** into each vial (STD, CTRL, sample), **mix thoroughly** by repeated inversion or several seconds on a vortex mixer.
3.	Incubate for **45 min** at room temperature (15-30 °C) on a **horizontal shaker**.
4.	Add **1250 µl assay buffer** (ASYBUF) into each vial and mix well.

2 x 50 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.

5.	**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.
6.	For the analysis in duplicate, take **2 x 50 µl** of the **derivatised standards/ controls/samples** out of the vials and add into the respective wells of the microtiter plate.
7.	Add **150 µl L-arginine antibody** into each well of the microtiter plate.
8.	Cover the strips tightly with foil and incubate **overnight at 2-8°C**.
9.	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.
10.	Add **100 µl conjugate** (CONJ) into each well.
11.	Cover the strips and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.

12.	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.
13.	Add **100 µl substrate** (SUB) into each well.
14.	Incubate for **8-12 min*** at room temperature (15-30 °C) in the **dark**.
15.	Add **100 µl stop solution** (STOP) into each well and mix well.
16.	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.

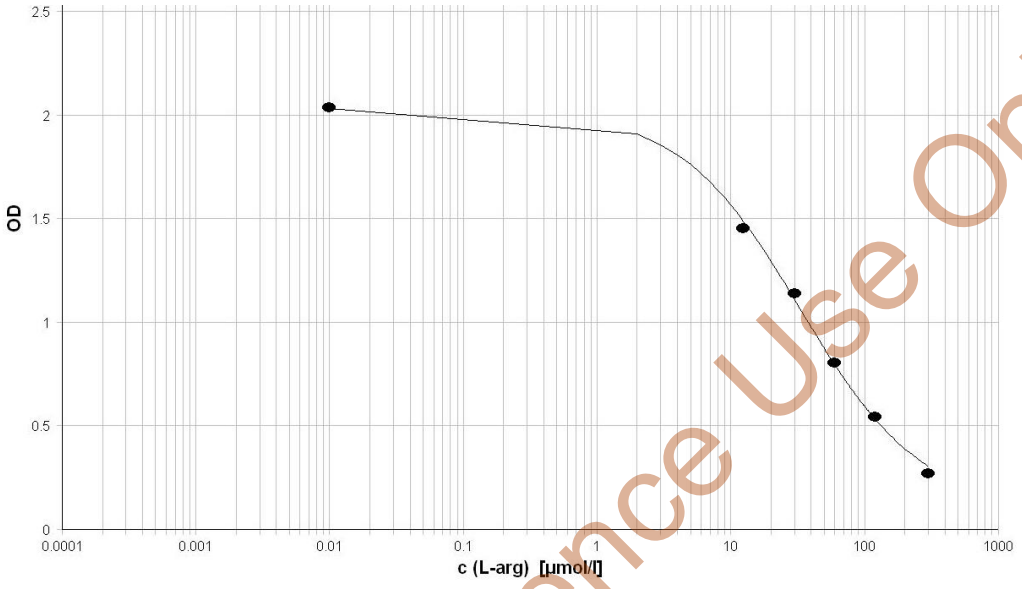
The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

EDTA plasma

Since the sample dilution is already considered in the standard curve, the dilution factor is 1.

In case an additional dilution factor is used, multiply the obtained result by the additionally used dilution factor.

In the following, an example of a standard 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 sample buffer (SAMPLEBUF) 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.

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-assay (n = 8)

Sample	L-arginine [µmol/l]	CV [%]
1	51.4	8.5
2	94.4	8.9

Inter-assay (n = 6)

Sample	L-arginine [µmol/l]	CV [%]
1	46.4	8.6
2	95.4	3.6

Spiking recovery

One plasma sample was spiked with different L-arginine concentrations and measured using this assay. The mean recovery rate was 103.3 % (n = 10).

spike [µmol/l]	expected [µmol/l]	measured [µmol/l]	recovery [%]
		49.5	
50	99.5	97.0	97.5
100	149.5	162.9	109.0

Dilution recovery

One spiked plasma sample was diluted with sample buffer. The mean recovery rate was 100.6 % (n = 6).

dilution	expected [µmol/l]	measured [µmol/l]	recovery [%]
		97.0	
1:1.3	72.8	66.7	91.6
1:1.5	64.7	64.6	99.8
1:2	48.5	49.5	102.1
1:3	32.3	35.2	108.8

Analytical sensitivity

The zero-standard was measured 32 times. The detection limit was set as B_0 - 2 SD and estimated to be 3.0 µmol/l.

Specificity

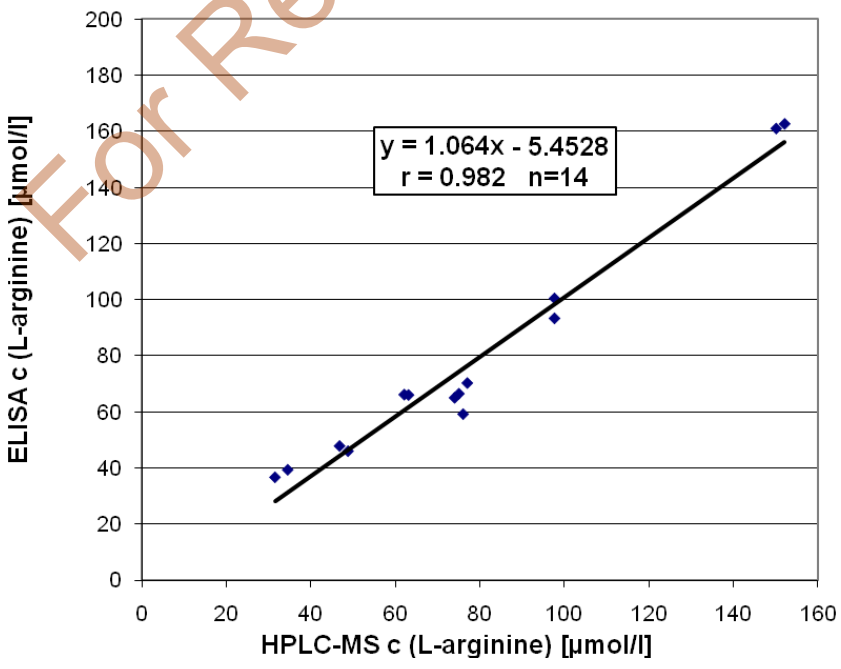
The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to L-arginine. The specificity is calculated in percent in relation to the L-arginine-binding activity:

ADMA < 0.06 %

SDMA < 0.006 %

Correlation with HPLC-MS

14 samples were measured with this ELISA and HPLC-MS. The correlation was r = 0.982.



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 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

Warning: Causes serious eye irritation

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.

- 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.

- *IDK*® is a trademark of Immundiagnostik AG.
- 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

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Literature using Immundiagnostik IDK® Arginine ELISA

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Used symbols:

	Temperature limitation	REF	Catalogue Number
RUO	For research use only	→REF	To be used with
	Manufacturer		Contains sufficient for <n> tests
LOT	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		