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Manual

α_1-microglobulin ELISA

For the in vitro determination of α_1-microglobulin in serum, plasma and urine

Valid from 2019-01-03

REF **K 6710**

Σ 96

+2 °C +8 °C

IVD

CE

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1. INTENDED USE

This Immundiagnostik assay is an enzyme immunoassay intended for the quantitative determination of α_1-microglobulin in serum, plasma and urine. For *in vitro* diagnostic use only.

2. INTRODUCTION

α_1-microglobulin, a glycoprotein heterogeneous in charge, was reported to occur both as a monomer of 31 kDa as well as a polymer of 90 kDa formed by a covalent binding with one of two alpha chains of the monomeric immunoglobulin A.

α_1-microglobulin is a protein with a small molecular weight produced in the liver. In healthy persons it is metabolised in the kidneys and only minor amounts can be detected in the urine.

Increased α_1-microglobulin concentration in serum is detected when the glomerular filtration rate is limited. In addition, when the ratio of total protein to the sum of albumin and α_1-microglobulin is disordered, a prerenal proteinuria should be suspected.

Indications

- Early diagnosis of inflammatory renal diseases
- Acute renal failure
- Renal and post renal proteinuria

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6710	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
K 6710	CONJ	Conjugate concentrate (rabbit-anti-α_1-microglobulin, peroxidase-labelled	1 x 400 µl
K 6710	STD	Standards, lyophilised (0, 0.019, 0.055, 0.166, 0.5, 1.5 mg/l)	2 x 6 vials
K 6710	CTRL1	Control, lyophilised (see specification for range)	1 x 1 vial
K 6710	CTRL2	Control, lyophilised (see specification for range)	1 x 1 vial
K 6710	NACL	0.9 % NaCl-solution, ready-to-use	2 x 100 ml

Cat. No.	Label	Kit components	Quantity
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	2 x 15 ml
K 0003.15	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.

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
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)
- 1 N NaOH-solution for storage of urine samples, if necessary
- 1 % BSA in PBS for dilution of urine samples, if necessary

* 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 4 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 **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **250 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at -20 °C until the expiry date stated on the label. Avoid repeated thawing and freezing.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (200 µl CONJ + 20 ml wash buffer). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is 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

Plasma and serum

Freshly collected plasma or serum can be stored for 2 weeks at 2–8 °C or for longer storage at -20 °C.

Plasma or serum samples must be diluted **1:500** with 0.9 % NaCl-solution (NACL) before performing the assay, e.g.

10 µl sample + **990 µl** NACL, mix well = **1:100 (dilution I)**

100 µl dilution I + **400 µl** NACL, mix well = **1:5 (dilution II)**

For testing in duplicates, pipette **2 x 10 µl** of each dilution II per well.

Urine

Urine should be adjusted to a pH of 6 to 8 with 1 N NaOH. Adjusted samples can be stored at 2–8 °C for 14 days. For longer storage, non-treated samples should be frozen at -20 °C.

Dilute all urine samples **1:20** with 1 % BSA in PBS, e.g.

50 µl urine + **950 µl** 1 % BSA in PBS, mix well.

For testing in duplicates, pipette **2 x 10 µl** of each dilution per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of α_1-microglobulin in serum, plasma and urine. The α_1-microglobulin in the samples is bound to an excess of polyclonal rabbit anti-α_1-microglobulin antibodies immobilised to the surface of the microtitre plate. After a washing step to remove all foreign substances, the quantification of the bound α_1-microglobulin is carried out by adding a peroxidase labelled antibody, which also binds to the α_1-microglobulin. The amount of converted peroxidase substrate is directly proportional to the amount of bound α_1-microglobulin. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. α_1-microglobulin, 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 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 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.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add **200 µl 0.9 % NaCl solution** (NACL) into each well.
3.	Add each **10 µl standards/controls/diluted samples** into the respective wells.
4.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
5.	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 firmly tapping the plate on absorbent paper.

6.	Add **200 µl conjugate** (diluted CONJ) into each well.
7.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
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 firmly tapping the plate on absorbent paper.
9.	Add **200 µl substrate** (SUB) into each well.
10.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
11.	Add **50 µl stop solution** (STOP) into each well and mix well.
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 range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* 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.

Serum and plasma

The obtained results have to be multplied with the **dilution factor of 500** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

Urine

The obtained results have to be multplied with the **dilution factor of 20** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

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

10. 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 sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik AG in-house studies of samples of apparently healthy persons, the following mean values were estimated.

Plasma or serum: < 60 mg/l

Urine: < 12 mg/l

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Inter-Assay (n = 17)

Sample	α_1-microglobulin [mg/l]	CV [%]
1	0.262	8.96

Analytical Sensitivity

The zero standard was measured 10 times. The detection limit was set as B_0 + 3 SD and estimated to be 0.006 mg/l.

Specificity

No cross reactivity to MPO and calprotectin was observed.

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic 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 thimerosal as bactericides. Sodium azide and thimerosal 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.

13. 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.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- 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 send to Immundiagnostik AG along with a written complaint.

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet