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Manual

α_2-Macroglobulin ELISA

For the in vitro determination of α_2-macroglobulin in urine, serum and plasma

Valid from 2019-07-23

REF **K 6610A**

Σ 96

+2°C +8°C

IVD CE

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1. INTENDED USE

The Immundiagnostik AG assay is intended for the quantitative determination of α_2-macroglobulin in urine, serum and plasma. For *in vitro* diagnostic only.

2. INTRODUCTION

Alpha-2-Macroglobulin (α2M) is one of the biggest plasma proteins, with a molecular weight of 650–900 kDa, depending on the degree of glycosylation. It consists of 4 identical subunits. α2M inhibits all known classes of endopeptidases by binding them and thereby blocking their active sites. The α2M-endopeptidase complex is then cleared rapidly from the circulation by the endocytotic proteinase clearance pathway. α2M also binds, transports and regulates many other molecules like defensins, myelin basic protein, and a host of other cytokines, growth factors, and hormones.

Measuring urinary proteins allows the diagnosis of proteinuria, which is defined as > 150 mg protein/day. Proteinuria can be divided into prerenal, renal (glomerular or tubular), and postrenal proteinuria depending on the localisation of the kidney damage. Differential diagnosis can be achieved by measuring certain marker proteins of different molecular weights. Very large proteins, such as α2M, are completely restricted from glomerular filtration in the kidneys. Thus, detecting α2M in urine is evidence of postrenal damage, when unfiltered serum proteins leak into the urine. Causes of postrenal damage are inflammation or hematuria as a consequence of renal stones or carcinomas.

Indications

- Detection and differentiation of proteinuria according to kidney damage localisation
- Differentiation of renal and postrenal hematuria

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6610A	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	1 x 100 ml
K 6610A	CONJ	Conjugate concentrate, rabbit anti α_2-Macroglobulin, peroxidase-labelled)	1 x 200 µl
K 6610A	STD	Standards, lyophilised (see specification for concentrations)	2 x 6 vials

Cat. No.	Label	Kit components	Quantity
K 6610A	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial
K 6610A	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
K 6610A	SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
K 0002.15	SUB	Substrate (Tetramethylbenzidine), ready-to-use	1 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, 3000 *g*
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* 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 con-

centration in the concentrate. 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 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 **500 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 for four weeks and used once after thawing.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until 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

Sample storage

Plasma and sera are stable at 2–8 °C for about 14 days. For long time storage we recommend –20 °C.

Sample dilution

Plasma and sera must be diluted **1:50 000** with **sample dilution buffer** (SAMPLEBUF). Dilution in three steps is recommended.

50 µl serum/plasma+ **950 µl** SAMPLEBUF, mix well = **1:20 (dilution I)**

20 µl of dilution I + **980µl** SAMPLEBUF, mix well = **1:1 000 (dilution II)**

20 µl of dilution II + **980µl** SAMPLEBUF, mix well = **1:50 000 (dilution III)**

For analysis, pipet 100 µl of dilution III per well.

Urine

Sample storage

Urine samples should be stored at -20 °C until the measurement. Alpha-2-macroglobulin in urine ist stable for 4 weeks at -20 °C.

Sample dilution

Urine samples must be diluted before the assay **1:5** with **sample dilution buffer** (SAMPLEBUF). For example:

100 µl urine+ 400 µl SAMPLEBUF, mix well (**1:5**).

For analysis, pipet **100 µl** of the **diluted urine** per well.

7. ASSAY PROCEDURE

Principle of the test

In a first incubation step, the α_2-macroglobulin in the samples is bound to polyclonal rabbit antibodies (in excess), which are immobilised to the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labelled anti α_2-macroglobulin antibody (POD-antibody) is added. After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The colour converts from blue to yellow. The intensity of the yellow colour is directly proportional to the concentration of α_2-macroglobulin in the sample. A dose response curve of the absorbance unit (optical density, OD) vs. concentration is generated, using the results obtained from the calibrators. α_2-macroglobulin, 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 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 each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	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.
5.	Add **100 µl conjugate** (diluted CONJ) into each well.
6.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
7.	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.
8.	Add **100 µl substrate** (SUB) into each well.
9.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
10.	Add **100 µl stop solution** (STOP) into each well and mix well.
11.	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 calibrator 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.

Plasma and serum samples

The obtained results have to be multiplied by the **dilution factor of 50 000** to get the actual concentrations.

Urine samples

The obtained results have to be multiplied by the **dilution factor of 5** to get the actual concentrations.

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

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution 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".

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

Plasma and serum: 1.3–3.0 g/l

Urine: < 0.18 mg/l; corresponds to 180 ng/ml

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy - Precision

Repeatability (Intra-Assay); n = 27

The repeatability was assessed with 2 serum and 2 urine samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [g/l]	CV [%]
Serum 1	3.92	2.4
Serum 2	2.15	7.8

Sample	Mean value [ng/ml]	CV [%]
Urine 1	163.65	2.3
Urine 2	84.84	3.5

Reproducibility (Inter-Assay); n = 13

The reproducibility was assessed with 2 serum and 2 urine samples under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [g/l]	CV [%]
Serum 1	3.82	12.7
Serum 2	1.85	11.6

Sample	Mean value [g/l]	CV [%]
Urine 1	156.94	5.0
Urine 2	82.97	4.5

Analytical sensitivity

The following value has been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB 1.611 ng/ml

Accuracy - Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, α2-macroglobulin spikes with known concentrations were added to 2 serum and 2 urine samples. The results below were obtained without consideration of the sample dilution factor.

Urine sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
19.13	200	219.13	199.25	90.93
	100	119.13	118.39	99.38
	50	69.13	64.02	92.61
34.74	200	234.74	223.79	95.34
	100	134.74	118.59	88.02
	50	84.74	80.46	94.95

Serum sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
17.29	200	217.29	191.95	88.34
	100	117.29	107.00	91.23
	50	67.29	64.04	95.17
31.77	200	231.77	227.51	98.16
	100	131.77	123.34	93.61
	50	81.77	80.40	98.33

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline

EP06-A with a serial dilution of a serum and a urine sample.

For α2-macroglobulin in serum and plasma, the method has been demonstrated to be linear from

16.13 to 297.07 ng/ml based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
Urine	1:5	297.07	297.07	100.00
	1:10	148.53	162.45	109.37
	1:12.5	118.83	112.15	94.38
	1:20	74.27	83.58	112.55
	1:25	59.41	62.28	104.83
	1:40	37.13	42.54	114.57
	1:50	29.71	34.74	116.93
Serum	1:10000	258.00	258.00	100.00
	1:20000	129.00	133.00	103.10
	1:40000	64.50	64.50	100.00
	1:80000	32.25	31.13	96.51
	1:160000	16.13	17.29	107.22

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 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.

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 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.

15. REFERENCES

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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