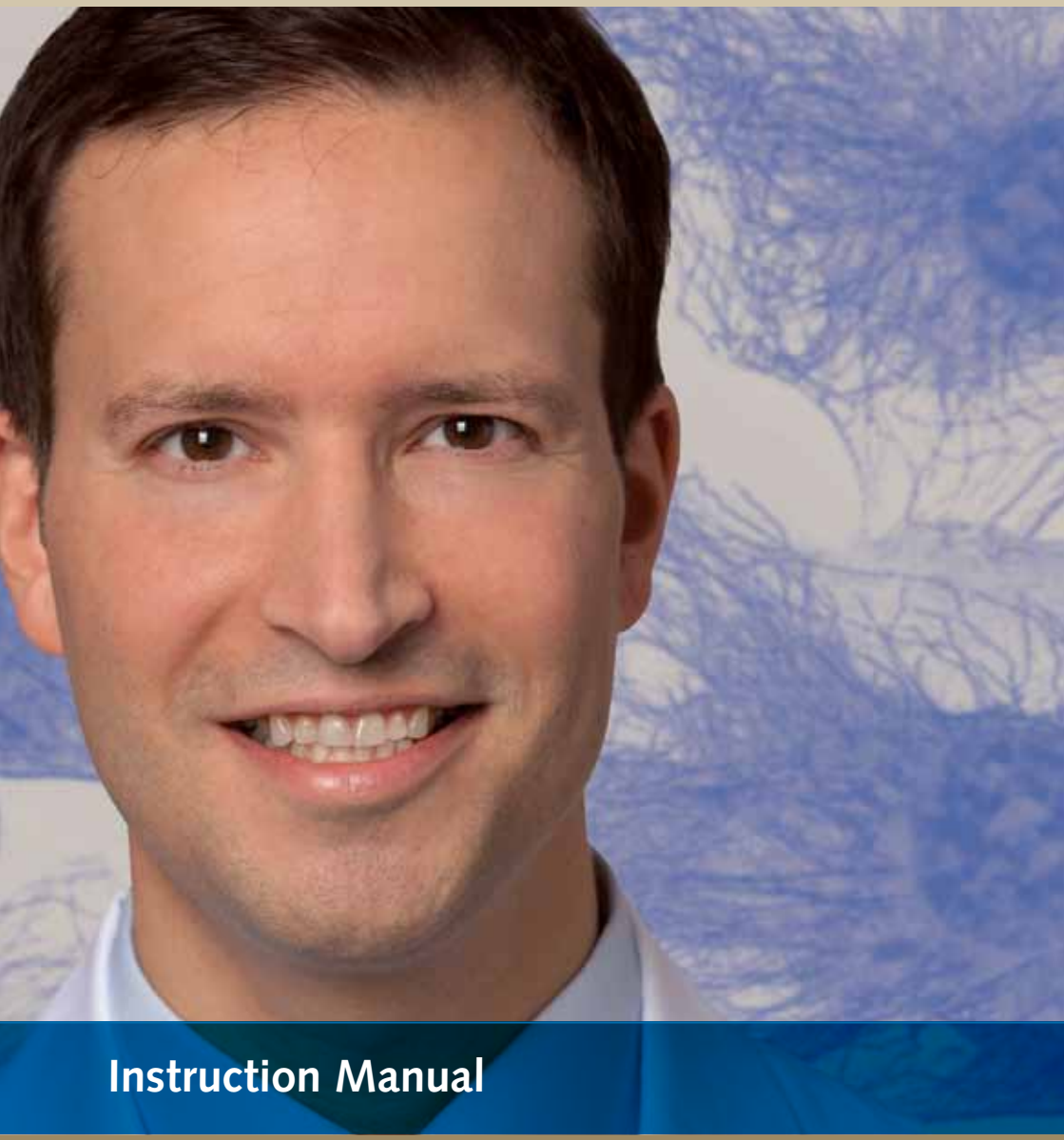
Human α2-Macroglobulin ELISA Kit



Instruction Manual

PromoKine

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Intended Use

This PromoKine ELISA is intended for the quantitative determination of α2-Macroglobulin in urine, serum and plasma. For *in vitro* research use only.

Summary and Explanation of the Test

Alpha-2-Macroglobulin (α2M) is one of the biggest plasma proteins, with a molecular weight of 650–900 kDa, depending on the degree of glyocsylation. 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 localization 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.

Indication

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

Principle of the Test

In a first incubation step, the α2-macroglobulin in the samples is bound to polyclonal rabbit antibodies (in excess), which are immobilized to the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled anti α2-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 color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of α2-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. α2-macroglobulin, present in the patient samples, is determined directly from this curve.

Material Supplied

Label	Kit Components	Quantity
PLATE	One holder with precoated strips	12 x 8 wells
STD	Standards/Calibrators (lyophilized)	2 x 6 vials
CTRL1	Control 1 (lyophilized)	2 x 1 vial
CTRL 2	Control 2 (lyophilized)	2 x 1 vial
WASHBUF	Wash buffer concentrate (10x)	1 x 100 ml
CONJ	Conjugate conc., (rabbit anti α-2-Macroglobulin, peroxidase-labeled)	1 x 200 µl
SAMPLEBUF	Sample dilution buffer, ready-to-use	2 x 100 ml
SUB	TMB substrate, ready-to-use	1 x 15 ml
STOP	Stop solution, ready-to-use	1 x 15 ml

Material Required but not Supplied

- Ultra-pure water*
- Precision pipettors calibrated to deliver 10-200 µl
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Microplate reader 450 nm

We recommend 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).

Preparation and Storage 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 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 solutions. The crystals must be redissolved at room temperature or in a water bath at 37 °C before dilution of the buffer solutions. date given on the label. 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 lyophilized calibrators (STD) and controls (CTRL)** are stable at 2–8 °C until the expiry date stated on the label. Before use, the standards and controls must be reconstituted with **500 µl of ultra-pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to ensure complete reconstitution. Reconstituted standards and controls **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 package label) when stored at **2–8 °C**.

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 color 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.The calibratiors and controls contain human source material which was tested and found to be non-reactive to HBsAg, anti-HIV-1/2, and anti-HCV. Since no method can offer complete assurance that hepatitis B virus, HIV-1/2, HVC or other infectious agents are absent, these reagents should be handled as if potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

Specimen Collection and Preparation

Plasma and serum

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

Plasma and sera must be diluted 1:50,000 with SAMPLEBUF (sample dilution buffer).

Dilution in three steps is recommended.

For example:

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

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

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

For analysis, pipet 100 µl of the final dilution (**dilution III**) per well.

Urine

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

For example:

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

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

Assay Procedure

Test procedure

- Bring all reagents and samples to room temperature (15–30 °C) and mix well.
- Mark the positions of STD /SAMPLE/CTRL (standards/sample/controls) on a protocol sheet.
- Take as many microtiter strips as needed from kit. Store unused strips covered 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. Please contact your supplier for details.
- We recommend to carry out the tests in duplicate.
- Note: Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. Carry out the assay with the actual manual delivered with the kit.

Test Procedure

1. Wash the precoated microtiter 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 **100 µl of STD/CTR L/SAMPLE** into the respective wells.
3. Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) shaking on a horizontal mixer.
4. 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.
5. Add **100 µl conjugate** (diluted CONJ) in each well.
6. Cover the strips and incubate for 1 hour at room temperature (15–30 °C).
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 **100 µl SUB (TMB substrate)** in each well.
9. Incubate for **10–20 minutes*** at room temperature (15–30 °C) in the dark.
10. Add **100 µl STOP** (ELISA stop solution) 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.

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

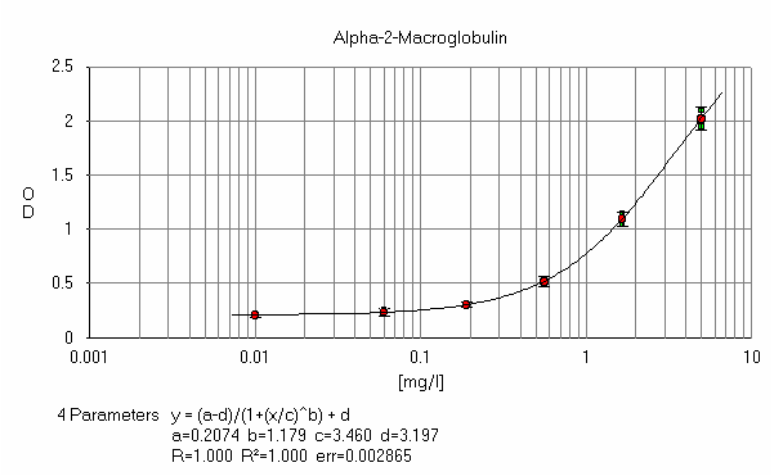
   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

   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 estimated plasma and serum concentration must be multiplied by the dilution factor of **50,000**.

Urine samples
The estimated concentration must be multiplied by the dilution factor of **5**.
In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

Typical calibration curve



Concentration [mg/l]	5	1.66	0.56	0.19	0.07	0
OD mean value	1.711	1.021	0.501	0.260	0.160	0.148

The data is only an example and cannot be used in place of data generations at the time of the assay. Generate your calibration curve for each new assay. Do not mix different lots.

Limitations

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this greater 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

Quality Control

PromoCell 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.

Performance Characteristics

Precision and Reproducibility

Intra-Assay Urine (n = 20)

Sample	α2-macroglobulin [ng/ml]	cV [%]
1	163.98	2.53
2	84.17	3.27

Intra-Assay Serum (n = 20)

Sample	α2-macroglobulin [ng/ml]	CV [%]
1	3.92	2.61
2	2.18	6.37

Inter-Assay Urine (n = 10)

Sample	α2-macroglobulin [ng/ml]	CV [%]
1	153.46	3.67
2	83.65	3.43

Inter-Assay Serum (n = 10)

Sample	α2-macroglobulin [ng/ml]	CV [%]
1	4.01	3.31
2	2.00	3.99

Spiking Recovery

Two samples were spiked with different α2-macroglobulin concentrations and measured using this assay (recovery n = 3).

Urine

Sample	unspiked Sample [ng/ml]	Spike [ng/ml]	α2-macroglobulin expected [ng/ml]	α2-macroglobulin measured [ng/ml]
A	19.1	200	219.1	199.3
		100	119.1	118.4
		50	69.1	64.0
B	34.7	200	234.7	223.8
		100	134.7	118.6
		50	84.7	80.5

Serum

Sample	unspiked Sample [ng/ml]	Spike [ng/ml]	α2-macroglobulin expected [ng/ml]	α2-macroglobulin measured [ng/ml]
A	17.3	200	217.3	192.0
		100	117.3	107.0
		50	67.3	64.0
B	31.8	200	231.8	227.5
		100	131.8	123.3
		50	81.8	80.4

Analytical Sensitivity

The Zero-standard was measured 20 times. The detection limit was set as B0 + 2 SD and estimated to be 1.68 ng/ml.

Dilution recovery

Two patient urine or serum samples were diluted with sample dilution buffer and measured with the assay. The results are shown below:

Urine

Sample	dilution	α2-macroglobulin expected [ng/ml]	α2-macroglobulin measured [ng/ml]
A	1:5	1485.30	1485.30
	1:10	742.65	812.30
	1:20	371.33	417.90
	1:40	185.66	212.70
B	1:5	1121.50	1121.50
	1:10	560.75	622.80
	1:20	280.38	347.40
	1:40	140.19	191.30

Serum

Sample	dilution	α2-macroglobulin expected [ng/ml]	α2-macroglobulin measured [ng/ml]
A	1:50 000	2.58	2.58
	1:100 000	1.29	1.33
	1:200 000	0.65	0.65
	1:400 000	0.32	0.31
B	1:50 000	1.74	1.74
	1:100 000	0.87	0.92
	1:200 000	0.44	0.48
	1:400 000	0.22	0.26

References

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- Rehman, Ahmed a., Haseeb Ahsan, and Fahim H. Khan. 2013. "Alpha-2-Macroglobulin: A Physiological Guardian." Journal of Cellular Physiology 228 (8): 1665–75.

General Notes on the Test and Test Procedure

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- Quality control guidelines should be followed.
- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- 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. PromoCell can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to PromoCell along with a written complaint.

Ordering Information

Product Name	Product Description	Size	Catalog Number
α2-Macroglobulin ELISA Kit, human	Human alpha2-Macroglobulin (a2-M) ELISA Kit	96 Tests	PK-EL-K6610

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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