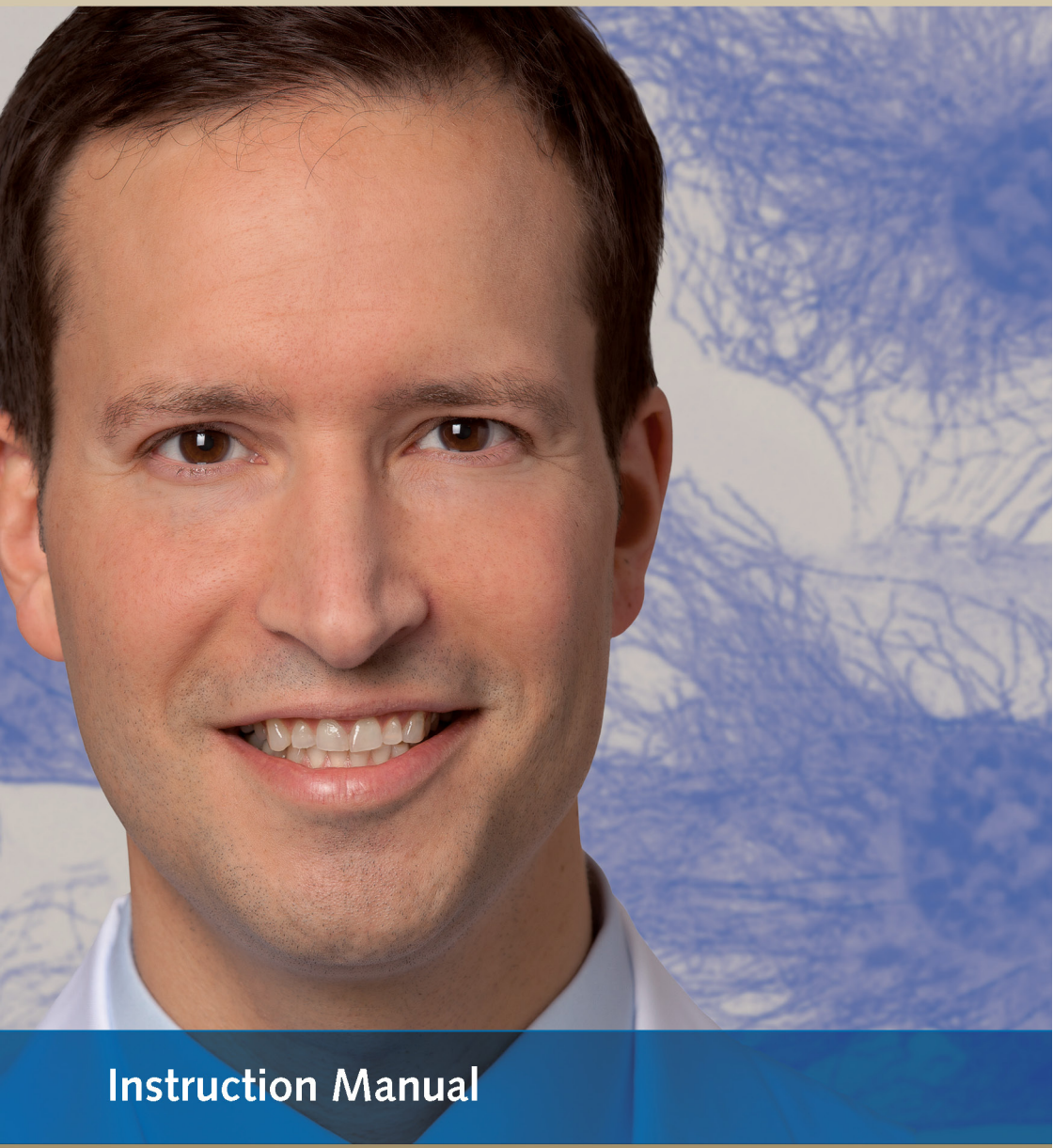
Human alpha1-Microglobulin ELISA Kit, human



Instruction Manual

PromoKine

Contents

Intended Use

The PromoKine Assay is intended for the quantitative determination of alpha1-Microglobulin in serum, plasma and urine. For *in vitro* diagnostic use only.

Summary and Explanation of the Test

Alpha1-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.

Alpha1-Mikroglobulin is a protein with a small molecular weight produced in the liver. In healthy persons it is metabolized in the kidneys and only minor amounts can be detected in the urine.

Increased **alpha1-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 **alpha1-microglobulin** is disordered, a **prerenal proteinuria** should be suspected.

Indications

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

Test Principle

This Enzyme-Linked Immunosorbent Assay (ELISA) allows the quantitative determination of human alpha1-Microglobulin from plasma, serum and urine.

The alpha1-Microglobulin in the samples is bound to an excess of polyclonal rabbit anti- alpha1-Microglobulin antibodies immobilized to the surface of the microtitre plate.

After a washing step to remove all foreign substances, the quantification of the bound alpha1-Microglobulin is carried out by adding a peroxidase labelled antibody, which also binds to the alpha1-Microglobulin. The amount of converted peroxidase substrate is

directly proportional to the amount of bound alpha1-Microglobulin and can be determined photometrically at 450 nm or at 405 nm if the extinction is out of range.

Material Supplied

Content	Kit Components	Quantity
PLATE	One holder with strips, break apart	96
WASHBUF	ELISA wash buffer concentrate (10x)	1 x 100 ml
CONJ	Conjugate (rabbit-anti-α1 microglobulin)	1 x 400 µl
STD	Calibrators, (0, 0.019, 0.055, 0.166, 0.5, 1.5 mg/l)	6 x 250 µl
CTRL1	Control 1, lyophilized	1 x 250 µl
CTRL2	Control 2, lyophilized	1 x 250 µl
NACL	0.9 % NaCl-solution, ready-to-use	200 ml
SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	2 x 15 ml
STOP	ELISA stop solution, ready to use	1 x 15 ml

Material Required but not Supplied

- Bidistilled (aqua bidest.) and sterile water
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 5-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 or 405 nm (reference wave length 620 or 690 nm)

Preparation and Storage of Reagents

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. 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.
- The **WASHBUF** (wash buffer concentrate) should be diluted with aqua dest. **1:10** before use (100 ml WASHBUF + 900 ml aqua dest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature at 37°C using a water bath **before dilution of the buffer solutions**. The **WASHBUF** (wash buffer concentrate) is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- The **STD** (Standards, Calibrators) and the **CTRL** (controls) must be reconstituted with **250 µl** aqua dest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted calibrators and control can be stored at –20 °C until the expiry date given on the label. Repeated thawing and freezing should be avoided.
- The **CONJ** (conjugate, POD-Antibody) must be diluted **1:100** in ELISA wash buffer (e.g. 200 µl CONJ + 20 ml WASHBUF). The antibody is stable at 2 -8 °C until expiry date given on the label. **Diluted antibody is not stable and could not be stored.**

Precautions

- 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.

- 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.
- Reagents should not be used beyond the expiration date shown on the kit label.

Specimen Collection and Preparation

Plasma or serum

Samples can be stored for two weeks at 4°C. They should be frozen when stored longer.
Dilute all plasma and serum samples **1:500 with NACL (0.9% NaCl)** (e.g. 10 µl sample + 990 µl 0.9% NaCl = 1:100 dilution; and then 100 µl from the 1:100 dilution + 400 µl 0.9% NaCl).

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)**.**

Assay Procedure

Procedural notes

- Do not interchange different lot numbers of any kit component within the same assay.
- Quality control guidelines should be observed.
- 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.
- The assay should always be performed according the enclosed manual.

Test procedure

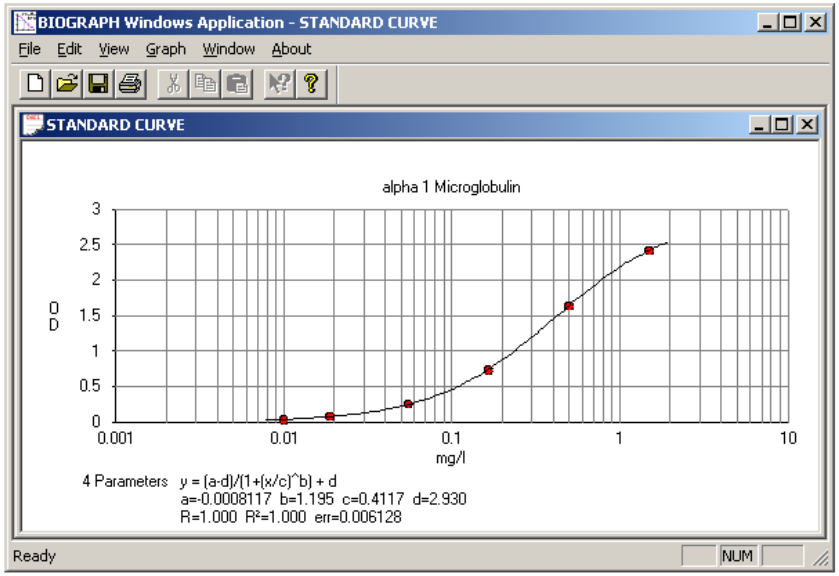
Carry out the tests in duplicate in the supplied microtitre plate.

1. Wash the cavities **5 x with 250 µl** of washing buffer.
2. Add **200 µl of NACL** (0.9% NaCl solution).
3. Add **10 µl** of **STD** (standard), **CTRL** (control) and **patient sample** into each well in duplicate
4. Cover the plate tightly and incubate for **1 hour at RT (18-26°C) shaking** on a horizontal mixer or for **2 hours at RT (18-26°C) without any shaking**.
5. Decant the contents of the wells and wash the cavities **5x with 250 µl** of washing buffer.
6. Add **200 µl pre-diluted conjugate**.
7. Incubate for **1 hour** shaking on a horizontal mixer at room temperature.
8. Decant the contents of the wells and wash the cavities **5x with 250 µl** of washing buffer.
9. Add **200 µl SUB** (TMB-substrate solution).
10. Incubate for **10 - 20 minutes** at room temperature until colour differences are sufficient.
11. Add **50 µl of STOP** (stop solution) and mix shortly.
12. Determine **immediately** absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

Results

A calibration curve is constructed from the standards. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve. THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL algorithm is recommended.

Typical calibration curve



Concentration [mg/l]	0	0.019	0.055	0.166	0.5	1.5
OD mean value	0.025	0.076	0.250	0.732	1.638	2.414

The data is for demonstration only and cannot be used for the evaluation of test results.

Serum, Plasma

The result must be multiplied by **500** to calculate the concentration of the sample.

Urine

The result must be multiplied by **20** to calculate the concentration of the sample.

Performance Characteristics

Precision and reproducibility

Intra-Assay (n= 17)

Sample	α-1-Microglobulin Mean value [mg/l]]	VC [%]
1	0.262	8.96

Sensitivity

The detection limit was set as B0 + 3 SD and estimated to be 0.006 mg/l. The Zero-standard was measured 10 times.

Sample	α-1-Microglobulin Mean value [OD]	Standard deviation	Detection limit [mg/l]
1	0.0023	0.002	0.006

Cross reactivity

No cross reactivity with MPO and calprotectin was observed.

Limitations

Samples with alpha-1-Microglobulin levels greater than the highest calibrator should be further diluted and re-assayed.

Quality Control

The use of independent external controls is recommended for quality control purposes.

Reference values

Plasma or serum: < 60 mg/l
Urine: < 12 mg/l

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.
- 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 color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- All reagents in the kit package are for *in-vitro* diagnostics only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.
- Guidelines for medical laboratories should be observed.
- 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 shall be send to PromoCell together with a written complaint.

Ordering Information

Product Name	Product Description	Size	Catalog Number
α1-Microglobulin (α1-M) ELISA Kit, human	Human alpha1-Mikroglobulin (α1-M) ELISA Kit	96 Tests	PK-EL-K6710

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

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