α2-Macroglobulin ELISA

For the in vitro determination of α2-macroglobulin in urine, serum and plasma

Valid from 2016-09-16

REF K 6610

Σ 96

+2°C +8°C

IVD CE
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1. INTENDED USE
This Immundiagnostik assay is intended for the quantitative determination of α₂-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 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.

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

3. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Label</th>
<th>Kit components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 6610</td>
<td>PLATE</td>
<td>Holder with pre-coated strips</td>
<td>12 x 8 wells</td>
</tr>
<tr>
<td>K 6610</td>
<td>WASHBUF</td>
<td>ELISA wash buffer concentrate (10x)</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>K 6610</td>
<td>CONJ</td>
<td>Conjugate concentrate, (rabbit anti α₂-Macroglobulin peroxidase-labelled)</td>
<td>1 x 300 µl</td>
</tr>
<tr>
<td>K 6610</td>
<td>STD</td>
<td>Standards, lyophilised</td>
<td>1 x 6 vials</td>
</tr>
</tbody>
</table>
# Manual

## Cat. No. | Label | Kit components | Quantity
--- | --- | --- | ---
K 6610 | CTRL1 | Control, lyophilised | 1 x 1 vial
K 6610 | CTRL2 | Control, lyophilised | 1 x 1 vial
K6610 | NACL | 0.9% NaCl solution, ready-to-use | 1 x 25 ml
K 6610 | SAMPLEBUF | Sample dilution buffer, ready-to-use | 1 x 100 ml
K 6610 | SUB | TMB substrate (tetramethylbenzidine), ready to use | 2 x 15 ml
K 6610 | STOP | ELISA 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

- Ultra pure water*
- Calibrated precision pipettors and 10–1000 µl tips
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* 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).

### 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 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 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 lyophilized 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 each **250 µl of ultra pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to ensure complete reconstitution. **Standards and controls** (reconstituted STD and CTRL) can be stored at **-20°C until the expiry date stated on the label**.

- **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 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 of test package) when stored at **2–8°C**.

## 6. STORAGE AND PREPARATION OF SAMPLES

### 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 **1:2 000 with SAMPLEBUF** (sample dilution buffer). Dilution in three steps is recommended.

For example:

- 30 µl sample + 270 µl SAMPLEBUF, mix well: dilution I (1:10)
- 50 µl of dilution I + 450 µl SAMPLEBUF, mix well: dilution II (1:10)
- 50 µl of dilution II + 950 µl SAMPLEBUF, mix well: dilution III (1:20)
  
This results in a final dilution of **1:2 000**.

For analysis, pipet 10 µl of dilution III per well.

### Urine
Urine samples can be measured directly.
7. ASSAY PROCEDURE

Principle of the test
In a first incubation step, the $\alpha_2$-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 $\alpha_2$-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 color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of $\alpha_2$-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 standards. $\alpha_2$-macroglobulin, present in the 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/samples/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. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

<table>
<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>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.</td>
</tr>
<tr>
<td>2.</td>
<td>Add 200 µl 0.9 % NaCl solution (NACL) into each well.</td>
</tr>
<tr>
<td>3.</td>
<td>Add 10 µl standards/controls/samples into the respective wells.</td>
</tr>
<tr>
<td>4.</td>
<td>Cover the strips tightly and incubate for 1 hour at room temperature (15–30 °C) shaking on a horizontal mixer.</td>
</tr>
<tr>
<td>5.</td>
<td>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.</td>
</tr>
<tr>
<td>6.</td>
<td>Add 200 µl conjugate (diluted CONJ) into each well.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>7.</td>
<td>Incubate for <strong>1 hour</strong> at room temperature (15–30 °C) <strong>shaking</strong> on a horizontal mixer.</td>
</tr>
<tr>
<td>8.</td>
<td>Discard the contents of each well and wash <strong>5 times</strong> with <strong>250 µl wash buffer</strong>. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.</td>
</tr>
<tr>
<td>9.</td>
<td>Add <strong>200 µl substrate</strong> (SUB) into each well.</td>
</tr>
<tr>
<td>10.</td>
<td>Incubate for <strong>10–20 minutes</strong>* at room temperature (15–30 °C).</td>
</tr>
<tr>
<td>11.</td>
<td>Add <strong>50 µl stop solution</strong> (STOP) and mix well.</td>
</tr>
<tr>
<td>12.</td>
<td>Determine <strong>absorption immediately</strong> with an ELISA reader at <strong>450 nm</strong> 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 <strong>405 nm</strong> against 620 nm (or 690 nm) as a reference.</td>
</tr>
</tbody>
</table>

* The intensity of the color change is temperature sensitive. We recommend observing the color 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 estimated plasma and serum concentration must be multiplied by the dilution factor of 2000.

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

9. **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:

\[
\text{highest concentration of the standard curve} \times \text{sample dilution factor to be used}
\]

The lower limit of the measurement range can be calculated as:

\[
\text{Analytical sensitivity} \times \text{sample dilution factor to be used}
\]

10. **QUALITY CONTROL**

Immundiagnostik 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

Precision and reproducibility

Intra-Assay (n = 32)
The intra-assay variation of the Immundiagnostik α₂-macroglobulin ELISA was calculated from 32 determinations on each one of two samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>α₂-macroglobulin mean value [mg/l]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1125.53</td>
<td>6.20</td>
</tr>
<tr>
<td>2</td>
<td>676.92</td>
<td>7.96</td>
</tr>
</tbody>
</table>

Inter-Assay (n = 10)
The inter-assay variation of the Immundiagnostik α₂-macroglobulin ELISA was calculated from data on 2 samples obtained in 10 different assays by three technicians on two different lots of reagents over a period of three months.

<table>
<thead>
<tr>
<th>Sample</th>
<th>α₂-macroglobulin mean value [mg/l]</th>
<th>CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1937.12</td>
<td>9.23</td>
</tr>
<tr>
<td>2</td>
<td>1298.07</td>
<td>8.85</td>
</tr>
</tbody>
</table>

Spiking Recovery
Two samples were spiked with three different α₂-macroglobulin concentrations and measured with the assay (n = 3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Unspiked Sample [ng/ml]</th>
<th>Spike [ng/ml]</th>
<th>α₂-macroglobulin expected [ng/ml]</th>
<th>α₂-macroglobulin measured [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>344</td>
<td>521</td>
<td>865</td>
<td>736</td>
</tr>
<tr>
<td></td>
<td></td>
<td>415</td>
<td>759</td>
<td>730</td>
</tr>
<tr>
<td></td>
<td></td>
<td>293</td>
<td>637</td>
<td>585</td>
</tr>
<tr>
<td>B</td>
<td>176</td>
<td>521</td>
<td>697</td>
<td>571</td>
</tr>
<tr>
<td></td>
<td></td>
<td>415</td>
<td>591</td>
<td>540</td>
</tr>
<tr>
<td></td>
<td></td>
<td>293</td>
<td>469</td>
<td>420</td>
</tr>
</tbody>
</table>
Analytical Sensitivity
The sensitivity was set as $B_0 + 2$ SD. The zero-standard was measured 22 times.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha_2$-macroglobulin mean value [OD]</th>
<th>Standard deviation</th>
<th>Detection limit [mg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.019</td>
<td>0.011</td>
<td>0.058</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>$\alpha_2$-macroglobulin expected [mg/l]</th>
<th>$\alpha_2$-macroglobulin measured [mg/l]</th>
</tr>
</thead>
</table>

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 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.

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 analyzed 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 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 com-
  ponents 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 da-
  mage 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 Immundi-
  agnostik AG along with a written complaint.
15. REFERENCES


**Used symbols:**

- ![Temperature limitation](image)
- ![Catalogue Number](image)
- ![In Vitro Diagnostic Medical Device](image)
- ![To be used with](image)
- ![Manufacturer](image)
- ![Contains sufficient for <n> tests](image)
- ![Lot number](image)
- ![Use by](image)
- ![Attention](image)