β2-Microglobulin ELISA

Enzyme immunoassay for the quantitative determination of beta2-microglobulin in human serum, plasma and urine

REF ID59041

Σ 96

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Distributed by:

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1. INTENDED USE

The Assay is intended for the quantitative determination of beta-2-microglobulin in serum, plasma and urine. For in vitro diagnostic use only.

2. SUMMARY AND EXPLANATION OF THE TEST

β-2-Microglobulin is a light chain protein (11.8 kD) of the HLA-class-I antigens and is found on the cell membrane of all nucleated cells. This protein is metabolised extensively in the kidney. The serum concentration is influenced by the rates of synthesis and metabolism and is usually stable in healthy persons. Changes in the serum concentrations are indicative of disorders in glomerular and tubular functions.

Indications

- Early detection of a renal transplant rejection
- Assessment of the glomerular filtration rate (GFR)

3. PRINCIPLE OF THE TEST

This Enzyme-Linked Immunosorbent Assay (ELISA) allows the quantitative determination of β-2-microglobulin from plasma, serum and urine.

In this assay the β-2-microglobulin in the samples is bound to an available excess of polyclonal rabbit antibodies against β-2-microglobulin, which are immobilised to the surface of the microtitre plates. After a washing step, to remove all unbound substances, the quantification of bound β-2-microglobulin is carried out by adding an enzyme (horseradish peroxidase) labeled antibody (POD-antibody), which also binds to the β-2-microglobulin. The amount of bound enzyme is directly proportional to the β-2-microglobulin content. The substrate tetramethylbenzidine (TMB) is converted by the enzyme to a chromogenic compound, which can be determined photometrically at 450 nm (if extinction is out of range measure at 410 nm)
4. MATERIAL SUPPLIED

<table>
<thead>
<tr>
<th>Catalogue No</th>
<th>Kit Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>K 6210MTP</td>
<td>one holder with precoated strips</td>
<td>96</td>
</tr>
<tr>
<td>K 6210WP</td>
<td>ELISA wash buffer concentrate 10x</td>
<td>100 ml</td>
</tr>
<tr>
<td>K 6210PV</td>
<td>Sample dilution buffer, ready-to-use</td>
<td>1 x 100 ml</td>
</tr>
<tr>
<td>K 6210K</td>
<td>Conjugate (rabbit anti β-2-M, peroxidase-labelled), ready-to-use</td>
<td>2 x 15 ml</td>
</tr>
<tr>
<td>K 6210ST</td>
<td>Calibrators, lyophilized (0; 0.6; 1.2; 2.5; 5; 10 mg/l)</td>
<td>6 x 1 vials</td>
</tr>
<tr>
<td>k 6210ko</td>
<td>Control, lyophilized</td>
<td>1 x 1 vial</td>
</tr>
<tr>
<td>K 6210NaCl</td>
<td>0.9 % ige NaCl-solution, ready-to-use</td>
<td>25 ml</td>
</tr>
<tr>
<td>K 6710AC</td>
<td>ELISA stop solution, ready to use</td>
<td>1 x 7 ml</td>
</tr>
<tr>
<td>K 6210TMB</td>
<td>TMB substrate (Tetramethylbenzidine), ready-to-use</td>
<td>2 x 15 ml</td>
</tr>
</tbody>
</table>

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized water
- Precision pipettes calibrated to deliver 10-1000 µl
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Horizontal mixer
- Vortex-Mixer
- Microplate reader 450 nm
6. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, please make sure that the reagents are carefully stored as mentioned. Prepare just the appropriate amount necessary for the assay.

- The **ELISA wash buffer concentrate** should be diluted with aqua dest. **1:10** before use (add 900 ml aqua dest. to 100 ml concentrate). Crystals could occur due to high salt concentration. The crystals have to be resuspended **before dilution of the buffer solutions** using a water bath (37°C). The buffer concentrates are stable at 2-8°C until the expiry date stated on the label. Diluted solutions could be stored at 2-8°C for 1 month.

- The **calibrators** and **control** have to be reconstitute with **250 µl aqua dest** and then have to be diluted **1:50** with dilution buffer (for example 20 µl sample + 980 µl sample dilution buffer) before use. Diluted calibrators are not stable. Reconstituted calibrators are stable for 4 weeks at 2 – 8°C and for long term storage at -20 °C until the expiry date stated on the label.

- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at **2 – 8°C**.
7. PRECAUTIONS

- For in vitro diagnostic use only.
- The calibrators and controls contain human source material which was tested and found to be non-reactive to HBsAg, anti-HIV-1/2. Since no method can offer complete assurance that hepatitis B virus, HIV-1/2, HCV or other infectious agents are absent, these reagents should be handled as if potentially infectious.
- The stop solution consists of diluted Sulfuric 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 out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
- Reagents should not be used beyond the expiration date stated on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

All samples have to be diluted **1:50** with dilution buffer (for example 10 µl sample + 490 µl sample dilution buffer) before use.

**Plasma or serum**
Samples can be stored for two weeks at 4 °C. They should be frozen when stored longer. Dilute samples with a β-2-microglobulin content of more than 10 mg/l 1:10 with diluting medium.

**Urine**
Adjust the urine to a pH of 6 to 8 with 1 N NaOH and store samples at -20 °C until testing. Dilute samples with a β-2-microglobulin content of more than 10 mg/l 1:10 with sample dilution buffer.
9. **ASSAY PROCEDURE**

**Procedural notes**
- Do not interchange different lot numbers of any kit component within the same assay.
- The quality control guidelines should be observed.
- 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. The manufacturer can therefore not be held responsible for any damage.
- Carry out the assay with the actual manual delivered with the kit.

**Test procedure**
Wash the precoated microtiter plate 5 x with 250 µl ELISA wash buffer. Carry out the tests in duplicate.

1. Pipette **200 µl** of 0.9% NaCl solution into each well of the microtitre plate.
2. Add **10 µl** of the diluted standard solutions, control solutions and patient samples.
3. Incubate for **60 minutes**, shaking on a horizontal mixer, at room temperature.
4. Decant the contents of the plate and wash the cavities **5 x with 250 µl** of washing buffer solution.
5. Add **200 µl** of conjugate (POD-antibody) into each well.
6. Incubate for **15 minutes**, shaking on a horizontal mixer, at room temperature.
7. Decant the content of the plate and wash the cavities **5 x with 250 µl** of washing buffer solution.
8. Add **200 µl** of TMB-solution into each well.
9. Incubate for **5-15 minutes** at room temperature, shaking slightly, until colour differences are sufficient.

10. Add **50 µl** of stop solution and mix shortly.

11. Determine 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.
10. RESULTS

A calibration curve is constructed from the calibrators. 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

<table>
<thead>
<tr>
<th>Concentration [mg/l]</th>
<th>10</th>
<th>5</th>
<th>2.5</th>
<th>1.2</th>
<th>0.6</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD mean value</td>
<td>1.834</td>
<td>1.429</td>
<td>0.990</td>
<td>0.642</td>
<td>0.412</td>
<td>0.168</td>
</tr>
</tbody>
</table>

These data are for demonstration only and cannot be used instead of data obtained from the actual assay.
11. LIMITATIONS

Samples with levels greater than the highest standard should be diluted and re-assayed.

12. QUALITY CONTROL

The manufacturer recommends to use control samples for internal quality control.

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values are located outside the acceptable limits, the results for the patient sample may not be valid.

Normal range

Plasma or serum: < 2.5 mg/l
Urine: < 0.4 mg/l

13. PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit, defined as Bo + 2 SD, is 0.1 mg/l.

Linearity

The linearity of this assay was detected through dilution with assay buffer of material which contains β-2-microglobulin. The linearity is extended from 0.2 - 10 mg/l.

Recovery

Recovery studies were made with urine and plasma samples. The mean value for the urine samples is 93% and 98% for the plasma samples.
Precision and reproducibility

Through repeated measurements (n=12) of plasma and urine samples, containing β-2-microglobulin, the following results were obtained:

Intra-assay:

<table>
<thead>
<tr>
<th>Sample</th>
<th>β-2-M mean [mg/l]</th>
<th>Intra-Assay CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>5.7</td>
<td>9</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.1</td>
<td>11</td>
</tr>
</tbody>
</table>

Inter-assay:

<table>
<thead>
<tr>
<th>Sample</th>
<th>β-2-M mean [mg/l]</th>
<th>Inter-Assay CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>5.9</td>
<td>15</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.0</td>
<td>12</td>
</tr>
</tbody>
</table>
14. 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.

- The test components which are made of human serum are tested for Australia antigen and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory working should be observed.

- Reagents of the test package contain sodium azide as a bactericide. Contact with skin or mucous membranes has to be avoided.

- All reagents in the test package are to be used for in-vitro diagnostics only.

- The reagents should not be used after the date of expiry (see label on the test package).

- Single components with different lot numbers should not be mixed or exchanged.

- The guidelines for medical laboratories should be observed.

- 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 results of the test. The manufacturer can therefore not be held reliable for any damage resulting from this.
### Symbols of the kit components see MATERIALS SUPPLIED.

Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.

Voir MATERIEL FOURNI pour les symbôles des composants du kit.

Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.

Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.

Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.

Για τα σύμβολα των συστατικών του κιτ συμβουλεύετε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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<table>
<thead>
<tr>
<th>REF</th>
<th>LOT</th>
<th>CONC</th>
<th>LYO</th>
<th>IVD</th>
<th>CAUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.:</td>
<td>Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.:</td>
<td>Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato</td>
<td>Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Λυοφιλισμένο</td>
<td>In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Equipamiento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro.</td>
<td>Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione!</td>
</tr>
</tbody>
</table>

**LIABILITY**: Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.

Symbols Version 3.5 / 2008-10-01