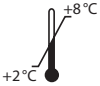
Manual

ß$_2$-Microglobulin ELISA

For the determination of ß$_2$-microglobulin in plasma, serum and urine

Valid from 2017-12-06

REF **KR6210**



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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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Table of Contents

1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of ß$_2$-microglobulin in serum, plasma and urine. For *research* use only.

2. INTRODUCTION

ß$_2$-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. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6210	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 6210	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
K 6210	NACL	0.9 % NaCl-solution, ready-to-use	1 x 25 ml
K 6210	CONJ	Conjugate, ready-to-use (rabbit-anti-ß$_2$-microglobulin, peroxidase-labelled)	1 x 25 ml
K 6210	STD	Standards, lyophilised (0; 0.6; 1.2; 2.5; 5; 10 mg/l)	1 x 6 vials
K 6210	CTRL1	Control, lyophilised (see specification for range)	1 x 1 vial
K 6210	CTRL2	Control, lyophilised (see specification for range)	1 x 1 vial
K 6210	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
K 6210	SUB	Substrate (tetramethylbenzidine), ready-to-use	2 x 15 ml
K 6210	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

- Ultra pure water*
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- 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.
- **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 concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. 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 1 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 **250 µl of ultra pure water**. Allow the vial content to dissolve for 10 minutes and mix thoroughly to ensure complete reconstitution. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 4 weeks. For long term storage up to 3 months they can be stored at -20 °C. Avoid repeated thawing and freezing.**
- 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

Sample storage

Serum and Plasma

Samples can be stored for two weeks at 2-8 °C. For longer storage, samples should be frozen at -20 °C.

Urine

Urines 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.

Dilution of samples

Plasma, serum and urine samples must be diluted **1:50** before performing the assay, mix well. For example

10 µl sample + **490 µl** sample dilution buffer (SAMPLEBUF).

Samples with a **ß$_2$-microglobulin content higher than 10 mg/l** should be **further diluted 1:10 with sample dilution buffer**.

For analysis, pipet **10 µl** of the dilution per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of ß$_2$-microglobulin.

In a first inicubation step, ß$_2$-microglobulin is bound to an immobilised antibody. Then a peroxidase-labelled anti-ß$_2$-microglobulin-antibody is added and a sandwich of capture antibody – ß$_2$-microglobulin – peroxidase-conjugate is formed. Tetramethylbenzidine (TMB) is used as peroxidase substrate. Finally, an acidic stop solution is added to terminate the reaction. The colour changes from blue to yellow. The intensitiy of the colour is directly proportional to the concentration of ß$_2$-microglobulin. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. ß$_2$-microglobulin, 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 the 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.

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 **200 µl 0.9 % NaCl-solution** (NACL) into each well.
3.	Add each **10 µl standards/controls/diluted samples** into the respective wells.
4.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
5.	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.
6.	Add **200 µl conjugate** (CONJ) into each well.
7.	Cover the strips and incubate for **15 min** at room temperature (15–30 °C) on a **horizontal shaker***.
8.	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.
9.	Add **200 µl substrate** (SUB) into each well.
10.	Incubate for **5–15 min**** at room temperature (15–30 °C) in the **dark**.
11.	Add **50 µl stop solution** (STOP) into each well and mix well.

12.	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 standard 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.

Serum, plasma and urine

Since the sample dilution is already considered in the calibration curve, the dilution-factor is 1.

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:

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

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

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Spiking Recovery

A plasma and a urine sample each were spiked with 5 mg ß$_2$-microglobulin and measured using this assay. The recovery-rate for plasma was 98 % and for urine 93 %.

Dilution recovery

The linearity of this assay was estimated by dilution of a ß$_2$-microglobulin sample in sample dilution buffer. The linearity extends from 0.2 to10 mg/l.

Precision and reproducibility

Intra-Assay (n = 12)

The reproducibility of two results in one measurement series was evaluated. A plasma and a urine sample were analysed 12 times by one person using the ß$_2$-microglobulin ELISA.

Sample	ß$_2$-microglobulin [mg/l]	CV [%]
urine	5.7	9
plasma	1.1	11

Inter-Assay (n = 12)

The reproducibility of two results at different days was evaluated. A plasma and a urine sample were analysed at different days by different persons using the ß$_2$-microglobulin ELISA.

Sample	ß$_2$-microglobulin [mg/l]	CV [%]
urine	5.9	15
plasma	1.0	12

Analytical Sensitivity

The detection limit was set as B_0 + 2 SD and estimated to be 0.1 mg/l.

12. 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 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 the 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.

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

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