



B2 Microglobulin

Enzyme immunoassay for the quantitative
determination of β_2 Microglobulin in human serum

Only for in-vitro diagnostic use



Product Number: DNOV066 (96 Determinations)

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1. INTRODUCTION

β_2 microglobulin is a component of MHC class I molecules, which are present on almost all cells of the body (red blood cells are a notable exception). It is an 11 kDa protein associated with the outer membrane of many cells including lymphocytes. Association with beta 2-microglobulin is generally required for the transport of class I heavy chains from the endoplasmic reticulum to the cell surface. Human Beta-2 Microglobulin (B2MG) is an 11.8 kDa protein identical to the light chain of the HLA-A, -B, and -C antigen.

β_2 microglobulin lies lateral to the $\alpha 3$ chain on the cell surface. Unlike $\alpha 3$, β_2 has no transmembrane region. Directly above β_2 (i.e. away from the cell) lies the $\alpha 1$ chain, which itself is lateral to the $\alpha 2$.

Beta 2-microglobulin is present in small amounts in serum, CSF, and urine of normal people, and to a much greater degree in the urine and plasma of patients with tubular proteinemia, renal failure, or kidney transplants.

In patients on long-term haemodialysis, it can aggregate into amyloid fibers that deposit in joint spaces, a disease known as dialysis-related amyloidosis.

2. INTENDED USE

Immunoenzymatic colorimetric method (ELISA) for quantitative determination of β_2 microglobulin in serum.

3. PRINCIPLE OF THE ASSAY

The enzyme immunoassay allows the quantitative determination of β_2 microglobulin from serum. In this assay, the β_2 microglobulin in the samples is bound to an available excess of monoclonal antibodies against β_2 microglobulin, which are immobilized to the surface of the microtiter wells. After a washing step to remove all foreign substances, the quantification of bound β_2 microglobulin is carried out by adding an enzyme (horseradish peroxidase or HRP) labelled 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 (TMB) is then converted to a chromogenic compound, which can be determined photometrically at 450 nm.

4. MATERIALS

4.1. Reagents supplied

- **Coated Wells:** 12 breakapart 8-well snap-off strips coated with anti- β_2 microglobulin antibodies; in resealable aluminium foil.
- **Stop Solution:** 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **HRP conjugate:** 1 bottle containing 22 ml of horseradish peroxidase labelled anti-mouse β_2 microglobulin antibodies.
- **TMB Substrate Solution:** 1 bottle containing 12 ml 3, 3', 5, 5'-tetramethylbenzidine (H_2O_2 -TMB 0.25g/l) (avoid any skin contact).
- **Serum Diluent:** 1 bottle containing 100 ml phosphate buffer (50 mM, pH 7.4) and BSA (1 g/l)
- **Wash Solution 50x conc.:** 1 bottle containing 15 ml NaCl 9 g/l and Tween 20 1 g/l
- **β_2 microglobulin standards:** 6 bottles, 0.5 ml each. They have the following concentrations:

Standard 0:	0 μ g/ml
Standard 1:	0.5 μ g/ml
Standard 2:	2 μ g/ml
Standard 3:	5 μ g/ml
Standard 4:	10 μ g/ml
Standard 5:	20 μ g/ml

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Manual or automatic equipment for rinsing wells
- Pipettes
- Vortex tube mixer
- Distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

The closed reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22...28°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti- β_2 microglobulin antibodies. Store at 2...8 °C. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.*

6.2. Anti- β_2 microglobulin -HRP Conjugate

The bottle contains 22 ml of a solution with anti- β_2 microglobulin antibodies conjugated with horse radish peroxidase. It is ready to use.

6.3. Standards

Each of the 6 vials contains 0.5 ml standard solution of the concentration mentioned in 4.1. The standards are ready to use. *After first use the standard solutions are still stable for another 6 months if stored at +4 °C.*

6.4. TMB Substrate Solution

The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stable for another 6 months if stored at 2...8 °C.*

6.5. Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C. *After first use stable until expiry date.*

6.6 Wash Solution

Dilute the contents of concentrated Wash Solution 50x to 750 ml with distilled or deionized water in a suitable storage container. Store at +2...+8°C until expiration date printed on the label of the concentrated wash solution.

7. SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Specimen can be stored at 2+8°C for at short time (max five days). For longer storage the specimen should be frozen. Avoid repeated freezing and thawing.

Pipette in a test tube:

Serum	10 μ l
Serum Diluent	1000 μ l

Mix gently.

7.1. Precaution

- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of β_2 microglobulin from 0.5 μ g/ml to 20 μ g/ml.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and standards should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well	(e.g. A1)	for the substrate blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(eg. F1+G1)	for standard 2
2 wells	(eg. H1+A2)	for standard 3
2 wells	(eg B2+C2)	for standard 4
2 wells	(eg. D2+E2)	for standard 5

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 5 µl standards and diluted samples into their respective wells. Add 200 µl serum diluent to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 30 min 37°C**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well 5 times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 200µl conjugate to each standard and sample, not to substrate blank. Cover wells with the foil supplied in the kit.
6. **Incubate for 30 min 37°C**
7. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well 5 times with 300 µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
8. Dispense 100 µl TMB Substrate Solution into all wells.
9. **Incubate for exactly 20 min at room temperature (22...28°C) in the dark.**
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
Any blue colour developed during the incubation turns into yellow.
11. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader **to zero** using the **substrate blank in well A1**.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard and patient sample in the distribution and identification plan.

Where applicable calculate the **mean absorbance values** of all duplicates.

9. RESULTS

9.1. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (Four Parameter Logistic).

Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in µg/ml.

9.2. Reference values

Healthy individuals are expected to have β₂ microglobulin values below 2.0 µg/ml.

10. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of β₂ microglobulin for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is 6.3 %.

Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is 7.8 %.

11.2. Specificity

The cross reaction of the antibody are shown in the table:

Antigens	Concentration	% Cross-reactivity
Human IgG	25 g/l	0.00
Ferritin	10,000 ng/ml	0.00
PSA	10,000 ng/ml	0.00
AFP	10,000 ng/ml	0.00
CEA	10,000 ng/ml	0.00

11.3. Sensitivity

The lowest detectable concentration of β_2 microglobulin that can be distinguished from the zero standard is 0.1 $\mu\text{g/ml}$ at the 95 % confidence limit.

11.4. Hook effect

The β_2 microglobulin ELISA, a competitive enzyme immunoassay, shows no Hook Effect up to 300 $\mu\text{g/ml}$.

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!
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13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

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15. ORDERING INFORMATION

Prod. No.: DNOV066 B2 Mircoglobulin Determination (96 Determinations)

SCHEME OF THE ASSAY

B2 Microglobulin

Test Preparation

Prepare reagents and samples as described.
 Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
 Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	blank	Stand. 0	Stand. 1	Stand. 2	Stand. 3	Stand. 4	Stand. 5	Dil. Sample
Stand. 0	-	5 µl	-	-	-	-	-	-
Stand. 1	-	-	5 µl	-	-	-	-	-
Stand. 2	-	-	-	5 µl	-	-	-	-
Stand. 3	-	-	-	-	5 µl	-	-	-
Stand. 4	-	-	-	-	-	5 µl	-	-
Stand. 5	-	-	-	-	-	-	5 µl	-
Diluted Sample	-	-	-	-	-	-	-	5 µl
Serum diluent	-	200 µl	200 µl	200 ml	200 µl	200 µl	200 µl	200 µl
Cover wells with foil supplied in the kit Incubate for 30 min at 37 °C Wash each well five times with 300 µl diluted wash solution								
Conjugate	-	200 µl	200 µl	200 ml	200 µl	200 µl	200 µl	200 µl
Cover wells with foil supplied in the kit Incubate for 30 min at 37 °C Wash each well five times with 300 µl diluted wash solution								
TMB Substrate	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 20 min at room temperature (22...28°C) in the dark								
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm								

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