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Instruction for use
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ORG 5BM Beta-2-Microglobulin

Immuno-metric Enzyme Immunoassay for the quantitative determination of Beta-2-Microglobulin in urine or serum

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NAME AND INTENDED USE

Beta-2-Microglobulin is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of β_2 -microglobulin in human urine, serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of lymphatic diseases and kidney dysfunctions.

SUMMARY AND EXPLANATION OF THE TEST

Proteins passing the glomerular basal membrane of the kidney undergo differentiated filtering. The permeability is inversely proportional to the molecular weight (Albumin about 0.6 %, Myoglobin about 75 %). Nevertheless, only minimal quantities of protein are detectable in urine, because big quantities of protein are reabsorbed by the tubuli. Elevated glomerular protein permeability and high tubular plasma protein elimination can be differentiated by measuring the molecular weight distribution of the eliminated proteins.

The pattern of eliminated proteins in urine gives information about:

- elevated protein elimination
- prediagnosis of a kidney defect
- differentiation of proteinuria
- glomerular or tubular proteinuria

Diagnostically relevant proteins:

- IgG (mw 150 kD)
- α_1 -Microglobulin (mw 33 kD)
- retinol binding protein (mw 21 kD)
- albumin (mw 66 kD)
- β_2 -microglobulin (mw 12 kD)
- immunoglobulin light chains (Bence-Jones protein) (22 kD)

β_2 -microglobulin has a molecular weight of 12 kD and belongs to the light chain part of membrane bound HLA antigens. It consists of two polypeptide chains, a heavy chain with antigenic structures and a light chain.

The determination of β_2 -microglobulin in serum or plasma is an aid in the clinical assessment of activation of the cellular immune system and a tumor marker. β_2 -microglobulin urine values indicate renal filtration disorders.

β_2 -microglobulin is synthesized in the lymphatic system. In Multiple Myeloma, Morbus Hodgkin, chronic lymphatic Leukemia and other malignant Non-Hodgkin Lymphoma elevated β_2 -microglobulin concentrations are detectable due to elevated cell biosynthesis. In these cases β_2 -microglobulin levels are a helpful indicator for disease development and therapy estimation. Other diseases with activation of the cellular immune system induce an elevation of β_2 -microglobulin in serum, too.

In the kidney β_2 -microglobulin is filtered glomerularly and reabsorbed tubularly. The molecule is not stable in urine with acid pH-values for a long time. A measurement of β_2 -microglobulin in serum and urine allows a differentiation between an activation of the lymphatic system and a disturbance of the kidney function.

Indications:

- changing of the glomerular and the tubular filtration
- renal tubular damage by heavy metals (Cd, Hg)
- lymphatic diseases
- repulsion of a kidney transplantate

PRINCIPLE OF THE TEST

Highly purified anti-human- β 2-microglobulin antibodies are bound to microwells. β 2-microglobulin, if present in diluted serum, plasma or urin, bind to the respective antibody. Washing of the microwells removes unspecific components. Horseradish peroxidase (HRP) conjugated anti-human β 2-microglobulin immunologically detects the bound patient beta-2-microglobulin forming a conjugate/ β 2-microglobulin/antibody complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of β 2-microglobulin present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified anti-human- β 2-microglobulin IgG (rabbit, polyclonal). Ready to use.
6 vials, 1.5 ml each	β 2-microglobulin Calibrators (A-F) in a PBS/BSA matrix ($\text{NaN}_3 <0,1\%$ (w/w)) containing β 2-microglobulin: 0; 0.75; 1.5; 3; 6 and 12 $\mu\text{g/ml}$. Ready to use.
2 vials, 1.5 ml each	β 2-microglobulin Controls in a PBS/BSA matrix ($\text{NaN}_3 <0,1\%$ (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, $\text{NaN}_3 <0,1\%$ (w/w)), yellow. concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, PROCLIN 300 $<0,5\%$ (v/v)), (light red) containing polyclonal rabbit anti-human β 2-microglobulin IgG; labeled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, $\text{NaN}_3 <0,1\%$ (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 μl
- Vortex mixer
- Pipets for 10 μl , 100 μl and 1000 μl

- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect either morning urine or whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum and urine samples. This may result in variable loss of protein activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2 - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample Preparation

Dilute all urine samples 1:10 with sample buffer before assay. Therefore combine 100 µl of urine with 900 µl of sample buffer in a polystyrene tube. Mix well. Dilute all serum or plasma samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 1,000 µl of sample buffer in a polystyrene tube. Mix well. Calibrators and controls are ready to use and need not to be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate calibrators, controls and prediluted patient samples in duplicates.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA - SF: standards A to F
P1, P2... patient sample 1, 2 ...
C1: positive control
C2: negative control

2. Pipet **100 µl** of calibrators, controls and prediluted patient samples into the wells.
3. Incubate for 30 minutes at room temperature (20 - 28 °C).
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate solution into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.

10. Add **100 µl** of stop solution to each well of the modules at leave untouched for 5 minutes.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600 - 690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The ORGENTEC Beta-2-Microglobulin ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calculation of results

For Beta-2-Microglobulin ELISA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Serum samples can be read directly from the standard curve ! Urine results have to be divided by 10 after calculation !

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example

The figures below show typical results for Beta-2-Microglobulin ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators									
No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl.Conc.	CV %
ST1	A 1/A 2	0.027	0.024	0.025	0.10	0.10	0.10	0.00	8
ST2	B 1/B 2	0.539	0.524	0.532	0.79	0.77	0.78	0.75	2
ST3	C 1/C 2	0.911	0.910	0.910	1.4	1.4	1.4	1.5	0
ST4	D 1/D 2	1.484	1.527	1.506	3.0	3.2	3.1	3.0	2
ST5	E 1/E 2	1.965	1.903	1.934	6.5	5.8	6.1	6.0	2
ST6	F 1/F 2	2.204	2.210	2.207	11.4	11.6	11.5	12.0	0

Interpretation of results

In a normal range study with urine and serum/plasma samples from healthy donors the following ranges have been established with the Beta-2-Microglobulin test:

Urine samples:	0 - 0.3 µg/ml
Serum or Plasma samples:	0 - 3.0 µg/ml

It is recommended that each laboratory establishes its own normal and pathological ranges of urine levels.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments urine and serum samples with high β₂-microglobulin concentrations were diluted with sample buffer and assayed in the kit. The assay showed linearity over the full measuring range.

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay			Inter-Assay		
Sample No	Mean [µg/ml]	CV [%]	Sample No	Mean [µg/ml]	CV [%]
1	1.6	4.2	1	1.7	4.9
2	7.2	2.6	2	7.5	3.8
3	12.5	3.6	3	13.1	4.9

Sensitivity

The lower detection limit for β₂-microglobulin has been determined at 0.1 µg/ml.

Specificity

The anti-β₂-microglobulin antiserum (polyclonal, rabbit) used for coating of the microplate is highly specific for human β₂-microglobulin. No crossreactivities have been observed.

LIMITATIONS OF PROCEDURE

The Beta-2-Microglobulin ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL)

triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

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INCUBATION SCHEME

