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Instruction For Use
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US Market: For Research Use Only

ORG 5BM Beta-2-Microglobulin

NAME AND INTENDED USE

Beta-2-Microglobulin is an ELISA test system for the quantitative measurement of beta-2-microglobulin in human urine, serum or plasma. This product is intended for research use only.

SYMBOLS USED

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Consult instructions for use
	Keep away from sunlight
	Do not reuse
	Date of manufacture

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified anti-human-beta-2-microglobulin antibodies are bound to microwells.
The reaction is based on indirect enzyme immuno assay (ELISA) method with these steps:
beta-2-Microglobulin present in a patient sample binds to the antibody coated forming an antigen-antibody-complex. Washing of the microwells removes unbound unspecific serum and plasma components. During incubation with anti-beta-2-mikroglobulin enzyme-conjugate immunologically a conjugate/antibody/antigen complex is formed. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

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 %,Myoglobulin 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,
- differentiation of proteinuria,
- prediagnosis of a kidney defect,
- glomerular or tubular proteinuria

Diagnostically relevant proteins:

- IgG (mw 150 kD),
- albumin (mw 66 kD),
- ß1-Microglobulin (mw 33 kD),
- ß2-microglobulin (mw 12 kD),
- retinol binding protein (mw 21 kD),
- immunoglobulin light chains (Bence-Jones protein) (22 kD)

ß2-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 ß2-microglobulin in serum or plasma is an aid in the clinical assessment
of activation of the cellular immune system and a tumor marker. ß2-microglobulin urine values indicate renal filtration disorders.

ß2-microglobulin is synthesized in the lymphatic system. In Multiple Myeloma, Morbus Hodgkin, cronical lymphatic Leukemia and other malignant Non-Hodgkin Lymphoma elevated.
ß2-microglobulin concentrations are detectable due to elevated cell biosynthesis. In these cases ß2-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 ß2-microglobulin in serum, too.

In the kidney ß2-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 ß2-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, lymphatic diseases, renal tubular damage by heavy metals (Cd, Hg), repulsion of a kidney transplantate

CONTENTS OF THE KIT

ORG 5BM	▽E 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***B2M***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 µg/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 0.75 µg/ml, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 1.5 µg/ml, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 3 µg/ml, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 6 µg/ml, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 12 µg/ml, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
CONTROL −	1x 1.5 ml	Control negative, containing beta-2-microglobulin in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
DILUENT	15 ml	Sample Buffer PU, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human ß2-microglobulin antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Instruction for Use: ELISA Mini-CD
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

ORGENTEC ELISA assays are suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

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

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production.
  Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.
  We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for research use only.
- 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.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer PU: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute **urine** samples **1:10** before the assay: add 100 µl of urine to 900 µl sample buffer.
Dilute **serum** samples **1:100** before the assay: add 10 µl of serum to 990 µl sample buffer.
Use polystyrene tubes. Mix well. Note: Calibrators / Controls are ready to use.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µl** of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µl** of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
   The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

The concentration of patient samples may then be estimated from the calibration curve by interpolation.

Due to different dilution, urine results have to be divided by 10 after calculation !

PERFORMANCE CHARACTERISTICS

CALIBRATION

The assay system is calibrated against the international reference preparation WHO B2M for Beta-2-Microglobulin.

Measuring range

The calculation range of this ELISA assay is 0 - 12 µg/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 0 - 3 µg/ml Serum / 0 - 0,3 µg/ml Urine

Interpretation of results

normal	< 3 µg/ml Serum /	< 0.3 µg/ml Urine
elevated	≥ 3 µg/ml Serum /	≥ 0.3 µg/ml Urine

Linearity

Patient samples containing high levels of beta-2-Microglobulin were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed µg/ml	Expected µg/ml	O/E [%]
1	1:100	11.4	11.4	100
.	1:200	5.6	5.7	98
.	1:400	2.7	2.9	93
.	1:800	1.3	1.4	93
2	1:100	9.6	9.6	100
.	1:200	4.6	4.8	96
.	1:400	2.2	2.4	92
.	1:800	1.1	1.2	92

Limit of detection

Functional sensitivity was determined to be: 0.1 µg/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean µg/ml	CV %
1	1.6	4.2
2	7.2	2.6
3	12.5	3.6

Inter-Assay		
Sample	Mean µg/ml	CV %
1	1.7	4.9
2	7.5	3.8
3	13.1	4.9

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

LIMITATIONS OF THE PROCEDURE

This assay 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges in patient samples should be regarded as recommendations only. Each laboratory should establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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