Beta-2 Microglobulina Latex (B2M) – Beta-2 Microglobulin Latex

For Konelab - Indiko® systems

Instructions for Use (IFU)

IVD CE

ORDERING INFORMATION

Format	Code	Composition
Kit 1 x 30 mL – 1 x 4 ml	REF B78282277	n°1 VIAL x 30 mL R:A n°1 VIAL x 4 mL R:B n°1 VIAL x 60 mL R.C

* in case of use on Indiko Analyser transfer the content into the additional empty vial

INTENDED USE

Diagnostic immunoturbidimetric latex test for the quantitative determination of Beta-2 Microglobulin in human serum, plasma and urine, using Konelab analyzers systems. All results must be interpreted in conjunction with the clinical picture. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

Beta-2 Microglobulin (B2M) is a protein with a molecular weight of 11,500 KD expressed on the surface of almost all human nucleate cells and is a component of the HLA antigenic complex. Due to its low molecular weight, free B2M filters through the renal glomerule, is reabsorbed (>99%) by the renal tubular cells and is completely degraded. The concentration of circulating B2M is influenced by the turnover of the nucleate cells, by renal function and by immunitary activation.

B2M is stable in the serum with a low concentration in normal subjects (0.1-0.2 mg/dL). An increase in the B2M serum concentration can be attributed to a reduction in glomerular filtration and/or an increase in its synthesis: it is present in patients affected by renal diseases such as diabetic nephropathies, and in organ rejection after liver transplant. The B2M concentration is correlated with several malignant tumors, chiefly multiple myeloma, lymphoma and with AIDS.

On the contrary, an increase in urinary B2M is associated with tubular insufficiency caused by various types of renal tubular diseases (inflammatory, degenerative, vascular), and this is the most important analyte to be studied in these types of pathological conditions. The normal urinary B2M content is very low (up to 0.15 mg/24 h). In view of the fact that B2M is spontaneously dissociated at acid pH, its detection in urine requires that the 24-hour urine collection be maintained at a pH above 6.5. At the diagnostic level, therefore, it is necessary to test for this protein in both the serum and the urine of the patient.

PRINCIPLE OF THE METHOD

Immunoturbidimetric method latex enhanced. Latex particles are activated with anti-human B2M antibodies (free and bound) through a covalent bond to increase the sensitivity and stability of the reagent. In the presence of B2M the suspension of sensitized particles agglutinates causing turbidity, determined spectrophotometrically, which is proportional to the B2M concentration in the sample.

Storage and stability

= **Storage temperature 2-8 °C**

If stored at 2-8°C avoiding direct light, the intact reagents remain stable until the expiration date, printed on the label. Slight variations in composition among batches will not affect test results.

Concentrations

Reagent A			
		Conc.	**U.M.**
Protein Buffer	TRIS pH 8.3	150	mmol/
	NaCL	100	mmol/
	EDTA	10	mmol/
	NaN_3	< 0,1	%
Reagent B			
Latex Particles coated with antibodies against B2M	NaN_3	< 0.1	%
Reagent C			
Sample Diluent	PBS	0,015	mol/L
	NaN_3	< 0,1	%

Materials included in the kit

Reagent as described above.

Necessary materials not included in the kit

Controls and calibrators.

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements. Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PREPARATION OF THE REAGENT

The Reagents are liquid, ready for use. After opening, the Reagents are stable until the expiry date if kept as indicated in "Storage and stability".

PROCEDURE

Quality Control

Serum: Use the Sclavo Diagnostics Int. Control Sera for specific Proteins Low REF B47182064 and High REF B47182065 for your quality control purposes at least once a day. Repeat the analysis also after calibration

Urine: Use the Sclavo Diagnostics Int. Controls: Urinary Proteins Control Low REF B47182223 and High REF B47182224 for your quality control purposes at least once a day. Repeat the analysis also after calibration.

Obtained values must be within the range of acceptability.

ANALYTICAL TECHNIQUE

For automatic procedures, consult the instruction manual and applicable notes for the Konelab® - Indiko®. analyzers. All applications not specifically approved by Sclavo Diagnostics Int. cannot be guaranteed in terms of performance and must be evaluated by the user.

Calibration

For calibration, use the Sclavo Diagnostics Int. Specific Proteins Single Level Calibrator REF B47182273, in accordance with methodology applying to Konelab® - Indiko® series for the use with serum and Urine Proteins Single Level Calibrator REF B47182222, in accordance with methodology applying to Konelab® - Indiko® series for the use with urine.

Traceability

The assigned value has been determined according to the IFCC using the reference material. The B2M value has been determined according to the NIBSC using the Reference Preparation for B2M "1st International Standard BETA-2 MICROGLOBULIN Code: B2M".

SAMPLE

Sample types and storage

Serum or plasma obtained by normal medical techniques can be used. No special preparation of the patient is necessary.

The samples must be pre-diluted 1:4 before analysis.

Urine: Urine samples are constituted of those which are normally delivered to the laboratory, and the test can be performed on either the early morning samples or the 24-h collection. No special preparation of the patient is necessary.

- To avoid B2M degradation in the urine, the pH must be kept at over 6.5.
- It is a frequent practice nowadays to perform the Creatinine titer on urine samples, as an indication of the dilution of the urine itself. This means that any urine sample can be tested, even if it is collected during the day, and the protein levels are reported in relation to the Creatinine titer (mg Protein/g Creatinine or mmol of Creatinine).
- The analytical method does not require any pre-treatment of the sample.

All samples must be brought to room temperature before testing. Strongly lipemic serum or plasma samples or those which present turbidity or presence of precipitates must be clarified by centrifugation (10 min. at 15,000 x g), before the test. Urine samples must be centrifuged at 2500 rpm for 15 minutes. Use the limpid supernatant for the test.

Calculation of results on Konelab® - Indiko® systems

Results are automatically calculated by analyzer based on the calibration curve. The analyzer automatically performs serial dilutions from a primary standard according to the method protocol. The calibration curve is obtained by interpolating the values obtained with an appropriate algorithm.

REFERENCE RANGE

Serum and plasma: 1,9 – 2,4 mg/L

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Urine :0 – 0.3 mg/L
0 – 0,18 mg/g Creatinine; 0 – 0,020 mg/mmole Creatinine.
As sex, age, geographical location and other factors can influence the normal values found in the population, each laboratory should determine its own normal, medium and pathological values for its own population.

CHARACTERISTICS/PERFORMANCE

Analytical Range – Antigen excess

The analytical range was tested using a strongly positive sample and serial dilutions in saline solution. The method guarantees a correct response throughout the minimal detectable measurement range and the calibrator higher concentration.
The present method does not show Antigen Excess until 400 µg/ml in serum-plasma and 70 mg/L in urine

Trueness

The Trueness of the analytical results has been determined according to the CLSI EP15-A2 guideline, using commercial control sera and urine samples. The data obtained are shown in the following table (confidence interval 95%).

SERUM				
Level	**Replicates**	**Mean (µg/mL)**	**Value**	**Recovery%**
Low	25	1.7	1.66	97.6
High	25	5.9	5.71	96.7
URINE				
Level	**Replicates**	**Mean (mg/L)**	**Value**	**Recovery%**
Low	25	0.61	0.52	85.2
High	25	1.42	1.22	85.9

Specificity

The method is 100% specific for human B2M

Interferences

The test method applied to serum and plasma samples was tested to check whether various substances added to the samples caused interference with the analytical result. Bilirubin 50 mg/dl, Ascorbic acid 50 mg/dl, EDTA 10 mM, Hemoglobin 500 mg/dl, Sodium citrate 1000 mg/dl, Sodium Heparin 40 mg/ml, Triglycerides 2% caused errors below 5%. The method was also tested in its application to urine samples, to test for interfering substances.
Levels of Urea 50 g/L, Ascorbic acid 5 g/L, Glucose 100 g/L, Hemoglobin 5 g/L, Uric Acid 20 g/L, human Albumin 20 g/L, cause errors inferior to 5%. Even if the urine samples are saturated with boric acid and thymol, the possible error remains below 5%. Higher concentrations were not tested. However, in view of the wide heterogeneity of potentially interfering substances and pharmaceuticals, for diagnostic purposes the results of this test must always be taken into consideration in conjunction with the clinical history of the patient, other clinical tests and medical investigations.

Precision

The Precision of the analytical results has been determined as Repeatability and Total Precision according to the CLSI EP15-A2 guideline, using commercial control sera. The data obtained are shown in the following table (confidence interval 95%).

Within-run Precision – Repeatability (serum – plasma)				
Level	**Replicates**	**Mean (mg/L)**	**DS**	**CV%**
Low	10	1.66	0.060	3.60
High	10	5.71	0.100	1.75
Total Precision (Within-lab Precision) (serum – plasma)				
Level	**Replicates**	**Mean (mg/L)**	**DS**	**CV%**
Low	10	2.08	0.098	4.71
High	10	8.15	0.171	2.10

Within-run Precision – Repeatability (urine)				
Level	**Replicates**	**Mean (mg/L)**	**DS**	**CV%**
Low	10	0.519	0.0101	1.94
High	10	1.223	0.0349	2.85
Total Precision (Within-lab Precision) (urine)				
Level	**Replicates**	**Mean (mg/L)**	**DS**	**CV%**
Low	10	0.56	0.029	5.19
High	10	1.44	0.066	4.55

Limits of sensitivity

The Sensitivity limit has been measured using serial dilutions of high concentrated urine. The smallest measurable concentration is in serum/plasma 0.12 µg/mL, urine 0. 034 mg/L.

Comparison between methods

The present method was compared with another commercially available method following the guidelines of the CLSI EP09-A2-IR, analyzing 77 human sera and 41 urine samples. The correlation data between the two methods are reported in the table below.

SERUM	
Parameter	**Estimation**
Intercept	-0.610
Slope	1.022
Correlation Coeff. (R)	0.991

URINE	
Parameter	**Estimation**
Intercept	0.157
Slope	1.0056
Correlation Coeff. (R)	0.974

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

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