

Human Beta-2 Microglobulin Urine Kit for use on the SPAPLUS®

For *in vitro* diagnostic use only

Product code: LK043.U.S

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FDA (USA) Information
Analyte name Beta-2 Microglobulin
Complexity Moderate



1 INTENDED USE

The kit is intended for the quantitative *in vitro* determination of beta-2 microglobulin (β2M) in human urine using the SPAPLUS analyser, to aid the diagnosis of active rheumatoid arthritis and kidney disease. The test result is to be used in conjunction with other clinical and laboratory findings.

2 SUMMARY AND EXPLANATION

β2M is a low molecular weight protein (11.8kD) found on the surface of most nucleated cells. It forms the light chain component of the histocompatibility antigen and is eliminated via the kidneys. Following filtration through the glomeruli it is reabsorbed and catabolised by the proximal tubular cells. Normally only trace amounts are excreted in the urine. However this is markedly increased in tubulo-interstitial disorders (refs 1-3).

3 PRINCIPLE

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

Latex-enhanced antibodies: Some antigen-antibody reactions do not form sufficiently large immune complexes to be detected turbidimetrically. If the antibody is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed with antigen is enhanced sufficiently to enable turbidimetric detection.

4 REAGENTS

- 4.1 β2M latex reagent:** Consisting of monospecific sheep antibody coated onto polystyrene latex. Preservative: 0.05% ProClin™*, 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.
- 4.2 β2M calibrator and controls:** Prepared from pooled human material and supplied in a stabilised liquid form. The calibrator has been referenced against the 1st International Standard for beta-2-microglobulin (Code: B2M), supplied by the National Institute for Biological Standards and Control (NIBSC; www.nibsc.ac.uk). Preservatives: 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine.
- 4.3 β2M Reaction Buffer:** Containing 0.099% sodium azide as a preservative.

*ProClin™ is a trademark of Rohm and Haas Corp., Philadelphia, PA.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and ProClin 300 and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are NOT interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The reagents, calibrators and controls may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The β2M Reagent and β2M Supplementary Reagent may be stored, uncapped, on the SPAPLUS analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

β2M is unstable in acidic urine so the following collection procedure is recommended: the patient should void the bladder, then drink at least 0.5L of water. A urine sample should be collected within one hour and the pH adjusted (with 1M NaOH) to pH6-8. Such samples can be stored for up to two days at 2-8°C and for two months at -20°C or below.

Repeated freezing of samples may result in deterioration and should be avoided by aliquoting prior to freezing.

8 METHODOLOGY

8.1 Materials provided

- 8.1.1 1 x 100 Tests Human β2M Urine Latex Reagent SPAPLUS
8.1.2 1 x Human β2M SPAPLUS Calibrator set 1-6 (6 x 1.0mL)
8.1.3 2 x 1.0mL Human β2M Urine SPAPLUS High Control
8.1.4 2 x 1.0mL Human β2M Urine SPAPLUS Low Control
8.1.5 1 x 100 Tests β2M Reaction Buffer SPAPLUS

8.2 Materials required but not provided

- 8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.

For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 44.

Item Name 44 U B2M		CALIBRATION		AutoFill
DATA INFORMATION		Type Logit 2 ▼		
Units	mg/L	Standard		
Decimals	2	1 #	4 #	
ANALYSIS		2 #	5 #	
Type	End ▼	3 #	6 #	
Main W.Length 1	600 ▼	NORMAL RANGE		
Sub W.Length	▼		MALE	FEMALE
Method			LOW	HIGH
CORR.		Serum	[]	[]
Y =	1 X + 0	Urine	[]	[]
		Plasma	[]	[]
		CSF	[]	[]
		Dialysis	[]	[]
		Other	[]	[]
Page : 1	Print	Hard Copy	Next Page	Save
			Return	

Item Name 44 U B2M		DATA PROCESS		ABSORBANCE LIMIT	
ASPIRATION		READ	START	END	
KIND	○ Single ● Double	MAIN	53	54	LOW -3.0
VOLUME μL		SUB	35	36	HIGH 3.0
SAMPLE		FACTOR		Reaction Check	
REAGENT1 VOL	165	Blank correction 1	○ ON ● OFF	CHECK POINT	
REAGENT2 VOL	80	ENDPOINT LIMIT 2.0	LOW -3		
Third mix ● OFF ○ ON		LINEAR CHECK (%) 0	HIGH 3		
R1 Blank	● Water - Blank	DILUTION			
		Diluent	● 99: Dil 1 ○ 100: Dil 2		
		Pre Dilution Rate	▼		
		Auto Rerun Dilution Rate High	20		
		Auto Rerun Dilution Rate Low	▼		
MONITOR		PROZONE CHECK			
0 LEVEL SPAN 1		FIRST	START	END	LIMIT (%) Min dOD
SPAN	3.0	[#]	[#]	[#]	[#] ○ Low ● High
		SECOND	[#]	[#]	[#] ○ Low ● High
		THIRD	[#]	[#]	[#] ○ Low ● High
Page : 2	Print	Hard Copy	Prev Page	Next Page	Save
				Return	

Item Name 44 U B2M

Auto Rerun SW
 On Off

Auto Rerun Range (Result)
 On Off
 Lower Higher

Serum Cal 1# Cal 6#
 Urine
 Plasma
 CSF
 Dialysis
 Other

Auto Rerun Condition (Absorbance)
Absorbance Range
 Lower On Off
 Higher On Off

Prozone Range On Off

Bottle Size (ml)
 24 Items 36 Items
 Reagent1 60 Reagent1 0
 Reagent2 R1 17.5 Reagent2 R1 0
 Reagent2 R2 9 Reagent2 R2 0

Page : 3 Print Prev Page Save Return

The calibrator (Standard #), prozone check (#) and Min dOD (#) values are found in the quality control certificate (SIN244.QC). Calibrator values on Page 1 should be entered in ascending order, i.e. the lowest value first. The prozone check and Min dOD values (#) should be entered on Page 2. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing the **Autofill** button is pressed after typing the value for calibrator 6 on page 1. View item parameter pages 3 and 4 to ensure correct value entry.

8.4.2 Special wash procedure for urine testing

Sample carry-over may occur from beta-2 microglobulin to **Freelite** assays. To protect against this, **Freelite** and beta-2 microglobulin samples must be run in separate batches. After running beta-2 microglobulin samples the onboard cuvettes must be cleaned using the SPAPLUS Weekly Wash Protocol before they can be reused with **Freelite** samples. Full instructions, parameters and bottles of wash fluid are supplied in SPAPLUS Weekly Wash Protocol and Bottles (IK050.S).

8.5 Measuring range

The measuring range of the β 2M Urine assay when using the standard 1/1 sample dilution is approximately 0.03 - 1.0mg/L. Where results are greater than the assay range samples should be manually diluted 1/10 and run at 1/20 to give an overall dilution of 1/200 (see section C of the SPAPLUS Reference guide).

Approximate measuring range at standard sample dilution (1/1):	0.03 – 1.0mg/L
Approximate measuring range at 1/20 sample dilution:	0.6 - 20mg/L

9 QUALITY CONTROL

- 9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPAPLUS Operation Manual.
- 9.2 Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to the local technical support organisation.
- 9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN244.QC). Sample results obtained should only be accepted if the control results are within $\pm 15\%$ of the concentration(s) stated.

10 LIMITATIONS

- 10.1 Turbidimetric assays are not suitable for measurement of lipaemic, haemolysed, turbid or microbially contaminated samples due to the unpredictable degree of non-specific scatter these sample types may generate. All samples must be centrifuged prior to assay. Unexpected results should be confirmed using an alternative assay method.
- 10.2 This assay has not been validated using paediatric samples.
- 10.3 Customers are strongly advised to run controls with every batch of samples being assayed. Should a control value be out of range against a stored curve, it is recommended that the control should be re-assayed using the same calibration curve. If the control value is still out of range the curve should be recalibrated and the controls re-assayed. If the control values are out of range against the new calibration curve check the instrument and parameters entered before repeating the assay. If problems persist, refer to the supplier.
- 10.4 Diagnosis cannot be made and treatment must not be given on the basis of β 2M measurements alone. Clinical history and other laboratory findings must be taken into account.
- 10.5 Antigen excess: The SPAPLUS monitors the reaction kinetics of each sample and compares the results to reaction limits set through extensive testing. Samples detected as being in excess are flagged with a "P" for prozone on the internal print out and are automatically remeasured at a higher sample dilution.

11 EXPECTED VALUES

Adult urine range

These ranges were obtained on the SPAPLUS analyser using normal donor urine. They are intended for guidance purposes only. The reference interval was calculated using non-parametric statistics and represents the central 95% of the population. It is strongly recommended that each user should generate their own β 2M reference range.

Beta-2 Microglobulin	Number (n)	Mean (mg/L)	Median (mg/L)	95 Percentile Range (mg/L)
Normal urine	116*	0.08	0.07	0.03-0.202

* 26 samples gave results below the assay range <0.03mg/L.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

Two studies were performed following CLSI *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2). The studies were performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers.

Beta-2 Microglobulin urine precision summary									
	Mean (mg/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Urine 1	0.04	0.002	4.8	0.004	9.5	0.003	7.2	0.005	12.8
Urine 2	0.19	0.003	1.5	0.018	9.6	0.000	0.00	0.018	9.8
Urine 3	0.75	0.005	0.7	0.021	2.8	0.041	5.5	0.046	6.2

12.2 Comparison

A correlation study was performed on 49 urine samples (24 normal, 25 from known renal impaired patients), using this kit on the SPAPLUS and the Binding Site β 2M assay on the BN™II. The study demonstrated agreement with the following Passing & Bablok fit over the assay range 0.03mg/L to 20mg/L:

$$y = 0.97x + 0.00 \text{ (mg/L)} \quad (y = \text{SPAPLUS}; x = \text{BN}^{\text{TM}}\text{II})$$

correlation coefficient $r = 0.993$ (calculated by linear regression)

BN™ is a trademark of Siemens Healthcare Diagnostics Inc.

12.3 Limit of Blank and Limit of Detection

The limit of blank is calculated as the mean blank (n = 60) plus 2 standard deviations. This is equivalent to a concentration of 0.006mg/L (neat).

The limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated as 0.012mg/L (n = 60) using a urine sample (neat).

The limit of quantitation for this assay is defined as the lowest point of the calibration curve which is 0.03mg/L (neat).

12.4 Linearity

The linearity of this assay has been confirmed using serially diluted urine samples, giving a regression equation of $y = 0.9929x - 0.069$ (mg/L), $R^2 = 0.9992$. (y = measured β 2M concentration, x = theoretical concentration) over the range of 0.8 - 18.3mg/L.

12.5 Interference

No significant assay interference by 200mg/L bilirubin, 238mg/L haemoglobin, 200mg/L ascorbic acid and 100mg/dL protein has been demonstrated at the minimum sample dilution (1/1) for urine.

Concentration	Ascorbic Acid	Bilirubin	Hb	Protein
Mean (mg/L)	0.07	0.55	0.07	0.06
% interference	+4.83	+0.61	+0.47	+1.23

12.6 Antigen excess

The antigen excess protection (P flag) on the SPAPLUS has been tested up to a level which is equivalent to 760mg/L.

13 BIBLIOGRAPHY

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3. Protein Reference Unit Handbook of clinical Immuno Chemistry. (1990) Ed. A Milford Ward, Publ. PRU Publications, Sheffield, 63-65.