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

For *in vitro* diagnostic use only

Product code: LK043.S

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

CE

1 INTENDED USE

The kit is intended for the quantitative *in vitro* determination of beta-2 microglobulin (β2M) in human serum and 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. Raised serum levels of β2M are associated with renal disease and rheumatoid arthritis. Elevated serum levels can also occur with systemic lupus erythematosus, malignant lymphoma and myeloma (refs 1-4).

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.

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

Serum samples: Use fresh or deep frozen serum samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to one week or for prolonged storage kept at -20°C or below (Ref.4). Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used.

Urine samples: 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 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 High Control*
8.1.4 2 x 1.0mL *Human β2M 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
8.2.5 SPAPLUS Weekly Wash Protocol and Bottles, Product Code IK050.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 14.

Item Name 14 B2M
DATA INFORMATION
Units mg/L
Decimals 2

ANALYSIS
Type End ▼

Main W.Length 1 600 ▼
Sub W.Length ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION AutoFill
Type Logit 2 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

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Item Name 14 B2M

ASPIRATION
KIND ○ Single ● Double
VOLUME µL
SAMPLE 7
REAGENT1 VOL 165
REAGENT2 VOL 80

Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ **ABSORBANCE LIMIT**
START END
MAIN 53 54 LOW -3.0
SUB 35 36 HIGH 3.0

FACTOR **Reaction Check**
Blank correction 1 ○ ON ● OFF
ENDPOINT LIMIT 2.0 CHECK POINT
LINEAR CHECK (%) 0 LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 20 ▼
Auto Rerun Dilution Rate High 40 ▼
Auto Rerun Dilution Rate Low 10 ▼

PROZONE CHECK #
START END LIMIT (%) Min dOD
FIRST [#] [#]
SECOND [#] [#] [#] ○ Low ● High
THIRD [#] [#] [#] ○ Low ● High

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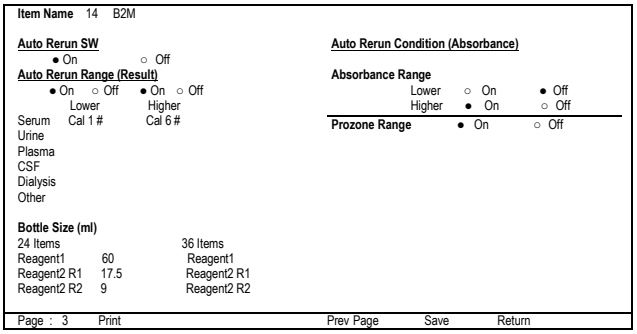
Item Name 14 B2M

Auto Rerun SW
● On ○ Off

Auto Rerun Range (Result)
● On ○ Off ● On ○ Off
Lower Higher
Serum Cal 1 # Cal 6 #
Urine
Plasma
CSF
Dialysis
Other

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

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ○ On ● Off
Higher ● On ○ Off

Prozone Range ● On ○ Off

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The calibrator (Standard #), prozone check (#) and Min dOD (#) values are found in the quality control certificate (SIN142.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 Running Urine Samples (<0.3mg/L)

If quantification of urine samples is required below 0.3mg/L the Pre Dilution Rate on *Item Parameter page 2* must be changed to neat; select the blank (neat) option from the drop down menu. The software will prompt you to input the Out-of-Range Table, click 'OK'. Click 'Previous Page' to return to item Parameter page 1; click 'AutoFill' for the measuring ranges to be updated. In page 3 the Auto rerun SW must also be switched to 'off' to prevent re-dilutions. In page 4 check the measuring ranges have been updated correctly and click Save.
For further details on parameter settings see Section C of the SPAPLUS Reference Guide.
Low level urine samples (<0.3mg/L) should be batched for testing at the neat sample dilution and the parameters must be changed back to the original Item 14 settings before any further serum and urine testing can be performed at the standard dilution 1/20.

8.4.3 Special wash procedure for urine testing.

Sample carry-over may occur from β2M urine samples to Binding Site Freelite assays. To protect against this, Freelite and β2M urine samples must be run in separate batches. After running β2M urine samples the onboard cuvettes must be cleaned using the SPAPLUS Weekly Wash Protocol before they can be re-used 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 β2M assay when using the standard 1/20 sample dilution is approximately 0.6 -20mg/L. The upper limit of the assay range using a sample dilution of 1/40 is 40mg/L. Where results are greater than the assay range samples should be manually diluted 1/10 and rerun at 1/20 to give an overall dilution of 1/200.

Approximate measuring range:	**0.6 - 20mg/L** 1/20 sample dilution
Approximate sensitivity - serum samples:	**0.3mg/L** 1/10 sample dilution
Approximate sensitivity - urine samples:	**0.03mg/L** Neat sample dilution

8.6 Interpretation of results

The results of this assay should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings including previous β2M results if available.

All immunoassays have the potential for antigen excess. In order to identify samples that are in antigen excess the SPAPLUS has the facility to monitor reaction kinetics. Samples that demonstrate unusual reaction kinetics will generate a P flag. Samples that have generated a P flag must be repeated at a higher dilution as described in section 8.5 (note: the SPAPLUS will carry out auto-dilutions up to 1/40). If upon repeat the sample gives a result that is considered implausible, the samples should be repeated at the initial dilution, reviewed and reported.

Refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser for further details of flag interpretation.

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 regular 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 (SIN142.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. 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 β2M measurements alone. Clinical history and other laboratory findings must be taken into account.

11 EXPECTED VALUES

Adult serum and urine ranges

These ranges were obtained on the SPAPLUS analyser using normal blood donor sera and normal donor urine. They are intended for guidance purposes only. The sera 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 β2M reference ranges.

Beta-2 Microglobulin	Number (n)	Mean (mg/L)	Median (mg/L)	95 Percentile Range (mg/L)
Normal sera	150	1.33	1.26	0.80-2.34
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 Serum Precision Summary									
	Mean (mg/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.97	0.02	1.8	0.06	5.8	0.05	5.6	0.08	8.2
Serum 2	4.94	0.03	0.6	0.10	2.1	0.12	2.4	0.16	3.3
Serum 3	16.43	0.09	0.6	0.31	1.9	0.62	3.8	0.70	4.3

An additional within run study was performed on three serum samples (Serum 4, 5, and 6) using one reagent lot and 20 measurements per sample (1/40 sample dilution).

Beta-2 Microglobulin serum within-run			
	Mean (mg/L)	Within-run	
		SD	CV %
Serum 4	20.74	0.35	1.7
Serum 5	31.81	0.65	2.0
Serum 6	36.53	0.62	1.7

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

Serum
A correlation study was performed on 103 serum samples (31 normal serum and 72 clinical serum) using this kit on the SPAPLUS and the Binding Site β2M assay on the BN™II. The study demonstrated agreement with the following Passing & Bablok fit over the measuring range of 0.5mg/L to 36.8mg/L:

y= 1.02x - 0.11 (mg/L) (y = SPAPLUS; x = BN™II)

correlation coefficient r = 0.996 (calculated by linear regression)

Urine
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 β2M 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 (mg/L) (y = SPAPLUS; x = BN™II)

correlation coefficient r = 0.993 (calculated by linear regression)

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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

Serum
The linearity of this assay has been confirmed using serially diluted serum samples, giving a regression equation of y=0.9896x + 0.150 (mg/L), R^2=0.9996. (y = measured β2M concentration, x = theoretical concentration) over the range of 1.0 -18.2mg/L.

Urine
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 β2M concentration, x = theoretical concentration) over the range of 0.8 - 18.3mg/L.

12.5 Interference

Serum
No significant assay interference by 10722 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, or 4.8g/L haemoglobin has been demonstrated at the minimum sample dilution (1/10) for sera.

Concentration	Chyle	Bilirubin	Hb
Mean (mg/L)	1.22	2.35	2.31
% interference	+1.39	-1.81	-0.72

No significant interference was observed with a serum sample containing 600 IU/mL Rheumatoid Factor (RF).

Urine
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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