B2MG

cobas®

Tina-quant β2-Microglobulin (urine application)

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08047430190	Tina-quant β2-Microglobulin (140 tests)	System-ID 07 6864 2	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

08047545190	Calibrator β2-Microglobulin	Code 474	
08362785190	Control Set β2-Microglobulin	Level I: Code 144 Level II: Code 145	
04489357190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

B2MGU: ACN 231

For **cobas c** 502 analyzer:

B2MGU: ACN 8231

Intended use

Immunoturbidimetric assay for the quantitative in vitro determination of β2-microglobulin (B2MG) in human urine on **cobas c** systems.

Summary

B2MG is a low-molecular-weight protein with approximately 12 kDa. It is identical to the light chain of the major histocompatibility complex (MHC) antigens (HLA, A, B, C). Thus B2MG is expressed on the cell membrane of nearly all nucleated cells (exception: trophoblasts).[1,2] Lymphocytes are the major location of synthesis. B2MG is constantly released into the blood in small quantities. Due to its low molecular weight, it is rapidly filtered through the renal glomeruli. Thereafter, up to 99.9 % is reabsorbed by the proximal tubules.[3]

Drug nephrotoxicity causing severe, acute changes in tubular reabsorption and progressive renal diseases causing irreversible structural tubular defects impair tubular reabsorption of numerous smaller proteins including B2MG.[4] Thus, urinary B2MG is discussed as a marker for the diagnosis and monitoring of tubulointerstitial renal damage.[2] Excretion of B2MG is increased in Fanconi syndrome, a generalized dysfunction of the proximal tubules. Causes for acquired Fanconi syndrome include exposure to toxins and drugs.[5]

Elevated B2MG values may identify patients at higher risk of glomerular filtration rate (GFR) decline in other kidney diseases such as membranous nephropathy.[6] Furthermore, there is evidence that B2MG excretion is associated with acute allograft rejection in renal transplant recipients.[7]

Various assay methods are available for B2MG determination, such as radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), nephelometric immunoassays, and turbidimetric methods.[2] The Roche B2MG assay is based on the principle of immunological agglutination with latex reaction enhancement.

Test principle

Immunoturbidimetric assay.

Latex-bound anti-β2-microglobulin antibodies react with antigen from the sample to form antigen/antibody complexes which are determined turbidimetrically after agglutination.[8]

Reagents - working solutions

R1	TRIS/HCl buffer: 23 g/L, pH 8.2; NaCl: 19 g/L; EDTA: 1.3 g/L; preservative
R2	Latex particles coated with polyclonal anti-human β2-microglobulin antibody (rabbit); preservative

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

B2MG

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks

Diluent NaCl 9 %

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Urine.

Each urine sample must be centrifuged (10 minutes at approximately 3000 x g) prior to testing.[9]
B2MG is unstable in acidic conditions and degradation occurs at pH < 6 within 2 hours.[2,10] Thus, pre-analytical conditions are highly important. Since the degradation also takes place in the bladder, collection of a spot urine sample should not be performed in the morning due to a lower urine pH.[2]
A strict control of the urine pH after collection is required: urine samples must be adjusted to pH 7-9 by the addition of 1 N NaOH as soon as possible after receipt.[10]

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability in urine:	5 days at 15-25 °C
	14 days at 2-8 °C
	12 weeks at -20 ± 5 °C
	Freezing and thawing up to 2 times is allowed.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

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The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for urine

cobas c 311 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 12-26	
Wavelength (sub/main)	-/700 nm	
Reaction direction	Increase	
Units	mg/L (nmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	124 µL	–
R2	124 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	10 µL	100 µL
Increased	2 µL	–	–

cobas c 501/502 **test definition**

Assay type	2 Point End	
Reaction time / Assay points	10 / 18-38	
Wavelength (sub/main)	-/700 nm	
Reaction direction	Increase	
Units	mg/L (nmol/L)	
Reagent pipetting		Diluent (H_2O)
R1	124 µL	–
R2	124 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	2 µL	10 µL	100 µL
Increased	2 µL	–	–

Calibration

Calibrators	S1: H_2O S2: Calibrator β2-Microglobulin
Calibration mode	Linear
Calibration frequency	2-point calibration - after reagent lot change - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the WHO standard.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factor: mg/L x 84.7 = nmol/L

Limitations - interference

Criterion: Recovery within ± 0.1 mg/L (8.47 nmol/L) of initial values at a β2-microglobulin concentration of ≤ 1.0 mg/L (84.7 nmol/L) and within ± 10 % for samples > 1.0 mg/L.

Hemolysis: No significant interference up to a hemoglobin concentration of 1100 mg/dL.[11]

High dose hook-effect: No false result occurs up to a B2MG concentration of 240 mg/L (20328 nmol/L).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.2-5.8 mg/L (16.9-491 nmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:11 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 11.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.1 mg/L (8.5 nmol/L)
Limit of Detection	= 0.15 mg/L (12.7 nmol/L)
Limit of Quantitation	= 0.2 mg/L (16.9 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration human β2-microglobulin samples.

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Expected values

B2MG (urine), male: ≤ 0.300 mg/L (25.4 nmol/L)[14]

B2MG (urine), female: ≤ 0.183 mg/L (15.5 nmol/L)[14]

B2MG/creatinine (urine): ≤ 0.029 mg/mmol[14]

24 h urine: 33-363 µg[10]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (4 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	mg/L (nmol/L)	*mg/L (nmol/L)*	*%*
Control 1	2.50 (212)	0.0529 (4.48)	2.1
Control 2	5.50 (466)	0.115 (9.74)	2.1
Human urine 1	0.274 (23.2)	0.0252 (2.13)	9.2
Human urine 2	0.354 (30.0)	0.0221 (1.87)	6.2
Human urine 3	0.891 (75.5)	0.0271 (2.30)	3.0
Human urine 4	2.90 (246)	0.0566 (4.79)	2.0
Human urine 5	4.85 (411)	0.102 (8.64)	2.1
Intermediate precision	*Mean*	*SD*	*CV*
	mg/L (nmol/L)	*mg/L (nmol/L)*	*%*
Control 1	2.53 (214)	0.0597 (5.06)	2.4
Control 2	5.50 (466)	0.131 (11.1)	2.4
Human urine 1	0.274 (23.2)	0.0257 (2.18)	9.4
Human urine 2	0.354 (30.0)	0.0241 (2.04)	6.8
Human urine 3	0.891 (75.5)	0.0344 (2.91)	3.9
Human urine 4	2.90 (246)	0.0777 (6.58)	2.7
Human urine 5	4.85 (411)	0.114 (9.66)	2.3

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

β2-Microglobulin values for human urine samples obtained on a **cobas c** 311 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 120

Passing/Bablok[15]	Linear regression
y = 1.000x + 0.010 mg/L	y = 0.993x + 0.024 mg/L
τ = 0.965	r = 0.999

The sample concentrations were between 0.210 and 5.76 mg/L (17.8 and 488 nmol/L).

β2-Microglobulin values for human urine samples obtained on a **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Siemens BN ProSpec® analyzer (x).

Sample size (n) = 105

Passing/Bablok[15]	Linear regression
y = 0.972x + 0.091 mg/L	y = 0.943x + 0.182 mg/L
τ = 0.935	r = 0.993

The sample concentrations were between 0.232 and 5.74 mg/L (19.7 and 486 nmol/L).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution

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Tina-quant β2-Microglobulin (urine application)

GTIN Global Trade Item Number

CE 0123

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