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INSTRUCTIONS FOR USE

In Vitro diagnostic medical device,
for laboratory professional use only

3diag - B2m - TIA KIT

Beta-2 Microglobulin - serum+urine
KIT - for Turbidimetry
REF **TD-42535**
UDI-DI **08434477302107**

INTENDED USE

Automated quantitative determination of Beta-2 Microglobulin (B2m, B2µ), in human serum and urine, in patient risk population, by turbidimetric method on Clinical Chemistry Analyzers.
The measurement of Beta-2 Microglobulin levels in serum can be used as an aid in the diagnosis of autoimmune and renal diseases.
The measurement of Beta-2 Microglobulin levels in urine, can be used as an aid to mark a renal tubular dysfunction due to any cause or pathology.
Assay results should always be used and interpreted in conjunction with clinical history and clinical manifestations, and other In Vitro and In Vivo diagnostic tests. A diagnosis or treatment decision should not be made based solely on test results.

SUMMARY AND EXPLANATION

ß2 microglobulin (B2m, B2µ) is a protein of low molecular weight (11800 Da) which forms the light chain of antigens of Class I Major Histocompatibility Complex (MHC) and is therefore found in the membrane of most nucleated cells, and in particularly high concentrations on the surface of lymphocytes. In free form, B2m is found in both serum and urine of normal individuals.
Elevated serum levels of B2m are either due to an increased production or to a decrease in the glomerular filtration rate which determines its accumulation. The increased rate of synthesis may be caused by many different diseases, in particular those of the lymphoid immune system, hematologic malignancies, viral infections, autoimmune diseases and others. Reports indicate that the ratio B2m/Cystatin-C may be a good indicator of the level of lymphoproliferation by individualizing the increased level of B2m of a possible altered renal function.
B2m is hence not used as a specific diagnostic marker for any pathology in particular, but is rather widely used as a quantitative prognostic marker in many diseases.
B2m, in a similar fashion to other micro-proteins, is filtered by the glomerulus and in normal conditions is almost completely reabsorbed by the renal tubules.
Measurement of B2m concentrations in urine is a very sensitive marker of tubular dysfunction due to any cause or pathology. Although as a tubular marker it suffers from the twin problems of instability in acidic environments (pH<6) and variable production levels, it is widely used due to the availability of the assay in many laboratories.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

Reagents

- Antiserum Reagent: **REAG | Ab | B2m**
  REF RAB-020 100 test

Anti-human B2m antibodies bound to polystirene particles (in lot-dependent concentration).

- Reaction Buffer: **REAG | BUF | B2m**
  REF RBF-020 100 test

TRIS Buffer, with PEG.

Calibrators

- Level 1: **CAL | 1 | B2m**
  REF S1-021 CONT 1 ml
- Level 2: **CAL | 2 | B2m**
  REF S2-021 CONT 1 ml
- Level 3: **CAL | 3 | B2m**
  REF S3-021 CONT 1 ml
- Level 4: **CAL | 4 | B2m**
  REF S4-021 CONT 1 ml
- Level 5: **CAL | 5 | B2m**
  REF S5-021 CONT 1 ml
- Level 6: **CAL | 6 | B2m**
  REF S6-021 CONT 1 ml

The calibrators are human B2m solutions.

Controls

- Serum - High Level: **CONTROL | H | B2m**
  REF CHS-022 CONT 1 ml
- Serum - Medium Level: **CONTROL | M | B2m**
  REF CMS-022 CONT 1 ml
- Serum - Low Level: **CONTROL | L | B2m**
  REF CLS-022 CONT 1 ml
- Urine - High Level: **CONTROL | H | U-B2m**
  REF CHU-022 CONT 1 ml
- Urine - Medium Level: **CONTROL | M | U-B2m**
  REF CMU-022 CONT 1 ml
- Urine - Low Level: **CONTROL | L | U-B2m**
  REF CLU-022 CONT 1 ml

The calibrators are human B2m solutions.

General

As preservatives, all the components contain <0.1% Sodium Azide (NaN_3). Calibrator and controls also contain <0.02% Methylisothiazolone and <0.02% Bromonitrodioxane.
All the components are ready for use and require no preparation.
Before each use it is convenient that the components are homogenized, shaking them gently (capped with their original caps) avoiding the formation of foam or bubbles .
If necessary, carefully transfer the components to the containers and cups used by the analyzer, preventing leakage and foaming or bubbles.
Before use, it is always advisable to bring the components to their use temperature, waiting a while before using them.
The values of calibrators and controls are lot-dependent and are indicated in their table of values.

WARNINGS - PRECAUTIONS

Calibrators and Controls

CAUTION! Potential Biohazard

- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.

WARNING! H317 May cause an allergic skin reaction
Contains: 2-methylisothiazol-3(2H)-one

Precautionary statements:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P280 Wear protective gloves/protective clothing/eye protection/face protection/hearing protection.

P302 + P352 IF ON SKIN: Wash with plenty of water.

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

P501 Dispose of contents, containers and any other waste material in accordance with applicable local, regional and national regulations.

Samples

- Samples, which cannot be clarified by centrifugation, should not be used in turbidimetric or nephelometric assays as turbidity and particles can interfere with the determination.

General

- Sodium Azide is toxic. Even if at the concentrations present Sodium Azide is not harmful, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
  Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- There are safety data sheets (SDS) available in the *Documentation* section (select folders *Beta2-Microglobulin* and ~~*SDS*~~ - https://www.3diag.com/S02) of the website (www.3diag.com) or upon request to the Customer Support Service (🖃 support@3diag.com - ☎ +34 93 2448679).
- Do not use any material after its expiration date.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.
- Any serious incident that has occurred in relation to the device shall be reported by the user to the manufacturer and to the competent authority of the Member State in which the user and/ or the patient is established[1].

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the components may be altered.
- Properly stored and unopened, the components are stable until the expiration date indicated on the label.
- Indications of deterioration: Discard the components if a change in color or appearance is observed (e.g. formation of aggregates), they have been frozen or they have not been refrigerated for a long time, or the quality controls do not give the expected results.

Open Vial Stability

- Once opened, provided that they are handled with adequate precautions to avoid contamination, the shelf life of the components is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC.
- The indicated shelf life must be taken as a guideline given that, obviously, it depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

- Automatic Clinical Chemistry Analyzer, capable of running photometric assays at 600 nm, and accessories: reagent containers, cuvettes, etc..

SAMPLES

Serum and urine are the only type of samples validated for the assay.

Serum - Collection and Handling

The collection and handling of serum samples does not require special conditions, and the usual laboratory procedure for routine serum assays and/or the recommendations in the literature[2][3][4] can be used.

Serum samples with presence of fibrin, hemolyzed, lipemic or turbid, and frozen samples after thawing, should be centrifuged prior to testing.

Urine - Collection and Handling

Either a 24-hour urine collection aliquot or a random urine collection are usually used. The 24-hour volume is required to determine daily urinary excretion. For random collection, RBP results should be expressed relative to urinary creatinine to adjust the value for variabilities in urine concentration.

Prior to testing, urine samples must be centrifuged until a clear and transparent supernatant is obtained[5].

For the determination of specific proteins, centrifugation of urine samples at 3000[6]-5000[7] g for 10 minutes is the standard practice in the laboratory.

The pH should be adjusted between 6 and 8, since the B2m degrades rapidly if pH <6. It is recommended[8] to use 1M NaOH.

General - Collection and Handling

Samples, which cannot be clarified by centrifugation, should not be used in turbidimetric or nephelometric assays as turbidity and particles can interfere with the determination.

For transport, specimens must be packaged and labelled in accordance with applicable regulations and recommendations governing the transport of clinical specimens and potentially infectious substances.

General - Storage and Stability

For both sample types, serum and urine, in internal studies the following stability conditions were assesed:

- Room Temperature (+20...+25ºC): up to 8 hours
- Refrigerated (+2...+8ºC): up to 3 days
- Frozen (below -20ºC): up to 2 weeks

These internal studies and bibliography support the application of the general recommendations regarding stability for serum samples, of the *Clinical and Laboratory Standards Institute* (*CLSI*) guideline GP44-A4[3], for both sample types, serum and urine:

- If assays will not be completed within eight hours, serum should be refrigerated (2 to 8ºC), if assays are not completed within 48 hours, or the separated serum will be stored beyond 48 hours, serum should be frozen at or below -20ºC.
- Frozen samples should be thawed only once, at room temperature, and mixed by inversion 10 to 20 times (no foam formation). Frost-free freezers are not suitable for storage.

CLSI guideline[3] also establish that it is the responsibility of each laboratory to consult all available references or to carry out its own studies to determine its specific stability criteria.

PROCEDURE

To program and calibrate assays, follow the instructions for use of the analyzer used, with the recommended general parameters that are detailed below. If you need further information contact Customer Support Service (🖃 support@3diag.com - ☎ +34 93 2448679).

Assay Parameters

- Dispense and mix:
  - Sample/Control: 16 (diluted 1:30 for serum and 1:5 for urine)
  - Calibrator: 16 µl (diluted 1:30)
  - **REAG | BUF | B2m** 200
- Incubate a fixed time of about 5 minutes
- Dispense and mix:
  - **REAG | Ab | B2m** 40
- Read absorbance A1 (Blank) at 600 nm
- Incubate a fixed time of about 5 minutes
- Read absorbance A2 (End Point) at 600 nm
- Interpolate the absorbance increment (A2-A1) of the samples and controls in the curve obtained with the calibrators

Warnings - Results

- Samples with concentrations higher than the upper limit of the assay range should be analyzed again, manually prediluded with Saline in successive steps of 1:5 (recommended), or by programming a larger sample dilution in the analyzer, until recover a concentration close to the midpoint of the measurement range.
- The stability of the dilutions is limited to their immediate use.

Calibration Parameters

- Use the 6-level calibrators included in the kit
- If the analyzer allows it, it is recommended to program two replicates of each calibration point.
- The calibrations are Non-linear. For the calculation it is recommended to use a Spline, a Logit or a Polygonal adjustment.

Warnings - Calibration

- The use of calibration update options with a single calibrator level is discouraged.
- The calibration curves have a limited validity, which depends on the particular conditions of use.
  Assays should be recalibrated:
  - when a new lot of reagents, buffer, or diluent is used,
  - when established internal quality control procedures do not deliver the expected results, or
  - after performing maintenance operations on the analyzer.

PERFORMANCES OF THE METHOD

Detailed information on the characteristics and performances of assays is given in the Performance Reports, available in the *Documentation* section (select folders *Beta2-Microglobulin* and ~~*Technical Reports*~~ - https://www.3diag.com/T02) of the website (www.3diag.com) or upon request to the Customer Support Service (support@3diag.com - ☎ +34 93 2448679).
The reported data are typical results and should not be considered as assay specifications, as the results obtained on another analyzer or at another time may be different.

QUALITY CONTROL

To monitor performances, it is recommended to use the controls included in the kit.
The insertion of internal controls is recommended:

- in each analytical series,
- when using a new reagent cartridge from the same lot, and
- after performing a calibration.

Each laboratory should establish its own quality scheme and corrective actions if controls do not meet the assigned tolerances, based on the applicable government regulations, the homologation requirements, and the general published recommendations, such as the guideline C24[9] of the *Clinical and Laboratory Standards Institute* (*CLSI*), or others.
The reagents have been subjected to quality control checks and should react as described in these instructions. Therefore, as a general recommendation, in the event that controls do not give the expected results, the following should be done:

- repeat controls,
- if the deviation persists, repeat with new controls,
- if the deviation persists, calibrate again, and
- if the deviation persists, contact the Customer Support Service (support@3diag.com - ☎ +34 93 2448679), or fill the *Technical Support Form* in the *Technical Support* section of the website (www.3diag.com).

As a precaution, until the causes of the deviation have been identified and corrected:

- all reagents should be considered unreliable, and
- sample results should not be validated.

TRACEABILITY

Values are referred to the *1st International Standard for Beta2 Microglobulin* (*NIBSC* code: *B2M*) of the *WHO* (*World Health Organisation*)[10].
Bibliography states that when changing the method, it is advisable to carry out additional sequential measurements to establish new baseline values that allow monitoring the evolution of patients.

REFERENCE INTERVALS

It is always advisable for each laboratory to establish its own reference values.

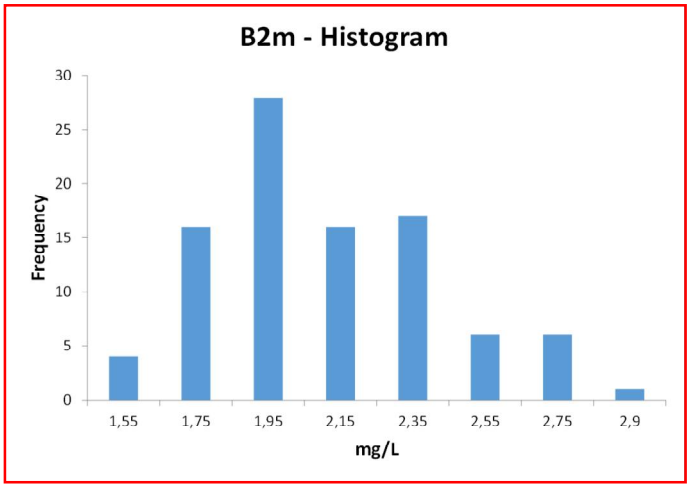
Serum

The bibliography reports variable reference values between publications, depending on the method used and the population analyzed:

- between 1.1 and 2.4 mg/l[11],
- between 1.21 and 2.70 mg/l[8],
- and between 1.16 to 2.52 mg/l for adults between 20 and 50 years and between 1.42 to 3.21 mg/l for adults older than 50 years[12].

Analyzing, by nephelometric method, serum samples of 94 presumably healthy adults from the Barcelona area (Spain), the following results have been obtained (see table and histogram):

units	mean	SD	range	95 percentile	90 percentile
IU/ml	143	22.8	96.4 - 200	98.6 - 190	114 - 186
mg/l	2.00	0.320	1.35 - 2.80	1.38 - 2.66	1.60 - 2.61



In view of the results, a concentration higher than about 2.65 mg/l, equivalent to about 190 IU/ml, can be taken as a significant value indicative of a B2m accumulation due to an increased production or to a decrease in the glomerular filtration rate.
The validity of the transference of this significant value has been verified analyzing, by turbidimetric method, serum samples of 20 presumably healthy adults from the Barcelona area (Spain). All obtained results were lower than this concentration.

Urine

The bibliography reports that an excretion greather than 0.37 mg/24h[11] or a concentration of 0.3 mg/l[8][13] can be considered significant.

The validity of the transference of this significant value has been verified analyzing, by turbidimetric method, urine samples of 20 presumably healthy adults from the Barcelona area (Spain). All the results obtained were lower than this concentration.

LIMITATIONS OF THE PROCEDURE

- Quantitation of specific proteins by nephelometry or turbidimetry may not be possible in lipemic sera due to extreme light scattering properties of the specimen. Turbidity and particles in the specimen may result in extraneous light scattering signals, resulting in variable specimen measurements.
- Samples containing circulating immune complexes (CIC)/ heterophilic antibodies can lead to erroneously increased or decreased results in immunoassays. Unexpected or inconsistent results should be confirmed using alternative methods.
- The product must be used as described in these instructions by suitably trained personnel. Any modification made to the assay and its necessary validation is the sole responsibility of the user, as well as its possible use in other analyzers.
- Samples from internal quality controls other than the recommended one, or from external quality controls, may give different results than those obtained by other methods, due to matrix effects. To evaluate the results it may be necessary to establish specific target values for the method.

SYMBOLS

In addition to the harmonized symbols provided on the EN ISO 15223-1:2021[14] norm, in the labels and instructions of use it has been used the complementary symbology proposed[15] by the *EDMA* (*European Diagnostic Manufacturers Association*).
The non-standard symbols meaning is detailed below.

UDI-DI	*UDI-DI* (Unique Device Identifier - Device Identifier)
REAG	Reagent
Ab	Antibody / Antiserum
B2m	Beta-2 Microglobulin
BUF	Buffer
CAL	Calibrator
n	Level n (n=1..6)
CONTROL	Control
H	High Level
M	Medium Level
L	Low Level
U-B2m	Beta-2 Microglobulin - Urine
CONT	Contents

BIBLIOGRAPHY

(1) "*Regulation (UE) 2017/746 of the European Parliament and of the Council* on In Vitro Diagnostic Medical Devices ", 5 April 2017.
(2) World Health Organization (WHO), WHO/DIL/LAB/99.1 Rev.2, January 2002: "*Use of Anticoagulants in Diagnostic Laboratory Investigations & Stability of Blood, Plasma and Serum Samples*".
(3) Clinical and Laboratory Standards Institute (CLSI), Doc. GP44-A4, May 2010: "*Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Test; Approved Guideline - Fourth Edition*".
(4) J.C. Dale: "*Preanalytic Variables in Laboratory Testing*" - Laboratory Medicine, Vol. 29, no. 9, Sept. 1998.
(5) Morales LJ., Ventura S., Solé E et al. - Comite de Comunicación de la Sociedad Española de Medicina de Laboratorio, SEQC[ML]: "*Muestras de Orina de 24 horas y Orina Reciente para la Medición de las Magnitudes Biológicas Más Comunes*", ISBN: 978-84-89975-52-1 (2017).
(6) "*Alpha-1-Microglobulin (A1M) - IMMAGE® Immunochemistry Systems Chemistry Information Sheet*", © Copyright 2017 Beckman Coulter, Inc..
(7) Bergón Jiménez E., Bergón Sendín M.: "*Uso del cociente cadenas kappa/cadenas lambda en orina para el estudio de la proteína de Bence Jones*", Química Clínica 1999; 18 (5) 266-270.
(8) *Mayo Clinic Laboratories* website (www.mayocliniclabs.com), date of consultation: 9th February 2020.
(9) Clinical and Laboratory Standards Institute (CLSI), September 2016: "C24 - *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions - 4th Edition*".
(10) NIBSC - National institute for Biological Standards and Control (www.nibsc.ac.uk): "*WHO International Standard - The 1st International Standard for Beta2 Microglobulin - NIBSC code B2M - Instructions for Use (Version 6.0, Dated 04/04/2008)*".
(11) *"Nomenclator de Laboratorio Clinico AEFA/AEBM"* - ISBN: 84-486-0117-3.
(12) "Application Note: General Guideline for Determination of Beta-2-Microglobulin in Serum/Plasma" - rev. 4-oct-16, DakoCytomation Denmark A/S, Glostrup, Denmark.
(13) Mingcheng Li et al.: "Significance of joint detection of urinary proteins with low molecular weight as prognostic indicators for early diagnosis of diabetic nephropathy", Int J Clin Exp Med 2016: 9(6): 11877-11882.
(14) International Organization for Standardization and European Committee for Standardization (CEN): "EN ISO 15223-1:2021 *Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements (ISO 15223-1:2021)*".
(15) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

17th November 2023.
Due to the continuous process of monitoring and improvement of the performance and safety of the products, the Instructions for Use are periodically updated. Therefore, users must ensure that they are working with the appropriate revision.
To facilitate this task, an electronic version of the Instructions for Use specific to each batch of the product is available in the *Documentation* section of the *TRIMERO Diagnostics* website (www.3diag.com), which the user can access by selecting the family and product type, or using the *Search by batch* option. Additionally, a *QR* code and a link to the appropriate folder are provided on the external labels and documentation accompanying the product.