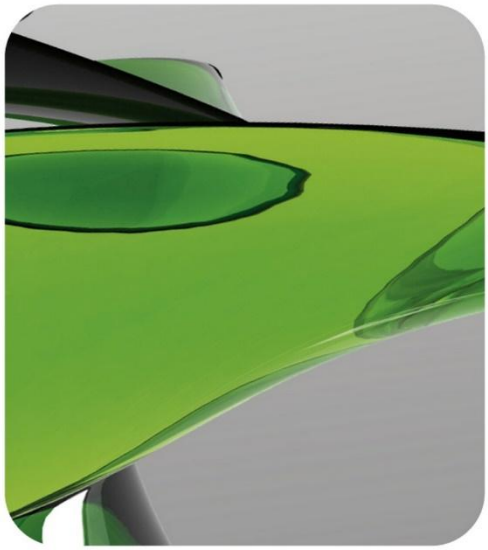
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THE DIAGNOSTIC TOOL THAT WORKS





AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA® ß-2 Microglobulin

Ref 3801



Instruction Manual

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1 Intended Use

***AESKULISA*® beta-2-Microglobulin** is a solid phase enzyme immunoassay employing highly purified anti-human-beta-2-Microglobulin antibodies for the quantitative detection of beta-2-Microglobulin in human serum and plasma.

The determination of beta-2-Microglobulin in serum or plasma is an aid in the clinical assessment of activation of the cellular immune system and a tumor marker.

2 Clinical Application and Principle of the Assay

beta-2-Microglobulin the light chain protein of HLA class I is located on the cell membranes on all cells containing nuclei. beta-2-Microglobulin provides a sensitive indicator for monitoring therapy and disease course in patients with multiple Myeloma, Hodgkin disease, chronic lymphatic leukemia, Non-Hodgkin lymphomas, and HIV infections. Other diseases with activation of the cellular immune system also induce an elevation of beta-2-Microglobulin in serum. Additionally, beta-2-Microglobulin is used as marker in the assessment of glomerular filtration rate, diagnostics and follow-up of tubulo-intestinal kidney damages and also in assessment of kidney function after kidney transplantation. The kit is designed for the quantitative measurement of beta-2-Microglobulin in human serum or plasma samples.

Indications:
- lymphatic diseases
- changing of the glomerular and the tubular filtration
- renal tubular damage by heavy metals (Cd, Hg)
- repulsion of a kidney transplantate

Principle of the test

Diluted samples are incubated in the microplates coated with anti-human beta-2-Microglobulin antibody. beta-2-Microglobulin, if present in the specimen, binds to the antibody. The unbound fraction is washed off in the following step. Afterwards anti-human beta-2-Microglobulin immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the bound beta-2-Microglobulin of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective beta-2-Microglobulin in the patient sample.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 0.75, 1.5, 3.0, 6.0, 12.0 µg/ml Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate: polyclonal rabbit anti-human beta-2-Microglobulin-IgG	1 x 15ml	Blue	Blue	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8 °C/35.6-46.4 °F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8 °C/35.6-46.4 °F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32 °C/68-89.6 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at 30 °C/86 °F for automated systems.

Never expose components to higher temperature than 37 °C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8 h, respectively stored tightly closed at 2-8 °C/35.6-46.4 °F up to 48 h, or frozen at -20 °C/-4 °F for longer periods

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8 °C/35.6-46.4 °F).

7.2 Pipetting Scheme

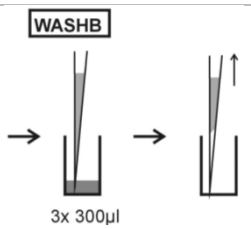
We suggest pipetting calibrators, controls and samples as follows:

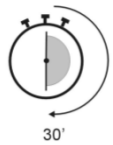
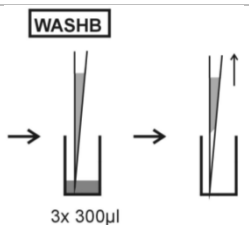
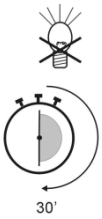
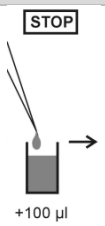
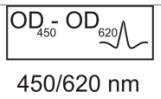
For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C
CalD: calibrator D
CalE: calibrator E
CalF: calibrator F
PC: positive control
NC: negative control
P1: patient 1
P2: patient 2
P3: patient 3

7.3 Test Steps

Step	*Description*	
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.	
2.	Use the following steps in accordance with quantitative interpretation results desired:	
	CONTROLS & SAMPLES	
3.		Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either: Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Patients diluted serum (P1, P2...)
4.		Incubate for 30 minutes at 20-32 °C/68-89.6 °F.
5.		Wash 3x with 300 µl washing buffer (diluted 1:50).

	CONJUGATE	
6.		Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32 °C/68-89.6 °F.
8.		Wash 3x with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.		Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32 °C/68-89.6 °F, protected from intense light.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

8 Quantitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in µg/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding protein concentrations expressed in µg/ml.

	Normal Range	Positive Results
serum or plasma	< 3.0 µg/ml	> 3.0 µg/ml

Example of a standard curve

Do NOT use this example for interpreting patient´s result

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0.0 µg/ml	0.049	3.1
0,75 µg/ml	0.480	2.1
1,5 µg/ml	0.845	1.7
3,0 µg/ml	1.335	1.6
6,0 µg/ml	1.720	0.3
12,0 µg/ml	2.023	3.1

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (µg/ml)
P 01	0.515/0.531	0.523	0.84
P 02	1.708/1.716	1.722	6.03

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

9 Technical Data

Sample material:	Serum or plasma
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32 °C/68-89.6 °F
Calibration range:	0-12.0 µg/ml
Analytical sensitivity:	0.1 µg/ml
Storage:	at 2-8 °C/35.6-46.4 °F use original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Analytical sensitivity

Testing sample buffer 30 times on ***AESKULISA***® **ß-2 Microglobulin** gave an analytical sensitivity of 0.1 µg/ml.

10.2 Specificity and sensitivity

The microplate is coated with highly purified rabbit anti-human beta-2-Microglobulin. No crossreactivities to other antigens have been found.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the hetero- geneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	Measured (µg/ml)	Expected (µg/ml)	Recovery (%)
1	1 / 100	11.9	12.0	99.2
	1 / 200	6.1	6.0	101.6
	1 / 400	2.9	3.0	96.7
	1 / 800	1.4	1.5	93.3
2	1 / 100	6.1	6.0	101.6
	1 / 200	3.1	3.0	103.3
	1 / 400	1.5	1.5	100.0
	1 / 800	0.7	0.75	93.3

10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-assay		
Sample No.	**Mean (µg/ml)**	**CV (%)**
1	8.0	3.9
2	4.6	4.1
3	2.3	2.8

Inter-assay		
Sample No.	**Mean (µg/ml)**	**CV (%)**
1	7.9	4.4
2	4.8	5.2
3	2.5	3.5

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in µg/ml.

11 Literature

Bernard A, Buchet JP, Roels H, Masson P, Lauwerys R. R. Renal excretion of proteins and enzymes in workers exposed to cadmium. Eur J Clin Invest. 1979 Feb;9(1):11-22.

Morell A, Riesen W. Serum beta 2-microglobulin, serum creatinine and bone marrow plasma cells in benign and malignant monoclonal gammopathy. Acta Haematol. 1980;64(2):87-93.

Camara NO, Nishida S, Silva MS, Pestana JO, Pereira AB, Sesso R, Pacheco-Silva A. Monitoring serum beta-2 microglobulin is useful for detecting patients with increased risk of acute rejection during reduction in immunosuppression. Transplant Proc. 1998 Dec;30(8):4158-9.

12 Regulatory Symbols

IVD	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
REF	- Numero d'ordine	- Cataloge number
	- Référence Catalogue	- Numéro de catálogo
	- Bestellnummer	- Αριθμός παραγγελίας
	- Número de catálogo	
LOT	- Descrizione lotto	- Lot
	- Lot	- Lote
	- Chargen Bezeichnung	- Χαρακτηρισμός παρτίδας
	- Lote	
UDI	- Identificatore univoco del dispositivo	- Unique Device Identifier
	- Identifiant unique de l'appareil	- Identificador único del dispositivo
	- eindeutige Produktidentifizierung	- Μοναδικό αναγνωριστικό συσκευής
	- Identificador único do dispositivo	
CE	- Conformità europea	- EC Declaration of Conformity
	- Déclaration CE de Conformité	- Declaración CE de Conformidad
	- Europäische Konformität	- Ευρωπαϊκή συμφωνία
	- Déclaracão CE de Conformidade	
Σ 96	- 96 determinazioni	- 96 tests
	- 96 tests	- 96 pruebas
	- 96 Bestimmungen	- 96 προσδιορισμοί
	- 96 Testes	
eIFU WWW.AESKU.COM	- Rispettare le istruzioni elettroniche per l'uso	- See electronical instructions for use
	- Voir les instructions d'utilisation électronique	- Siga las instrucciones electrónicas de uso
	- Elektronische Gebrauchsanweisung beachten	- Ακολουθήστε τις ηλεκτρονικές οδηγίες χρήσης
	- Seguir as instruções electrónicas de utilização	
	- Da utilizzarsi entro	- Use by
	- Utilise avant le	- Utilizar antes de
	- Verwendbar bis	- Χρήση μέχρι
	- Utilizar antes de	
+2°C +8°C	- Conservare a 2-8°C (35,6-46,4°F)	- Store at 2-8°C (35.6-46.4°F)
	- Conserver à 2-8°C (35,6-46,4°F)	- Conservar a 2-8°C (35,6-46,4°F)
	- Lagerung bei 2-8°C (35,6-46,4°F)	- Φυλάσσεται στους 2-8°C (35,6-46,4°F)
	- Conservar entre 2-8°C (35,6-46,4°F)	
	- Prodotto da	- Manufactured by
	- Fabriqué par	- Fabricado por
	- Hergestellt von	- Κατασκευάζεται από
	- Fabricado por	
CON +	- Controllo positivo	- Positive Control
	- Contrôle Positif	- Control Positivo
	- Positiv Kontrolle	- Θετικός ορός ελέγχου
	- Controlo positivo	
CON −	- Controllo negativo	- Negative Control
	- Contrôle Négatif	- Control Negativo
	- Negativ Kontrolle	- Αρνητικός ορός ελέγχου
	- Controlo negativo	
CAL	- Calibratore	- Calibrator
	- Etalon	- Calibrador
	- Kalibrator	- Αντιδραστήριο βαθμονόμησης
	- Calibrador	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
MP	- Micropiastra rivestita	- Coated microtiter plate
	- Microplaque sensibilisée	- Microplaca sensibilizada
	- Beschichtete Mikrotiterplatte	- Επικαλυμμένη μικροπλάκα
	- Microplaca revestida	
WASHB 50x	- Tampone di lavaggio	- Wash buffer
	- Tampon de Lavage	- Solución de lavado
	- Waschpuffer	- Ρυθμιστικό διάλυμα πλύσης
	- Solucão de lavagem	
SUB	- Tampone substrato	- Substrate buffer
	- Substrat	- Tampón sustrato
	- Substratpuffer	- Ρυθμιστικό διάλυμα υποστρώματος
	- Substrato	
STOP	- Reagente bloccante	- Stop solution
	- Solution d'Arrêt	- Solución de parada
	- Stopreagenz	- Αντιδραστήριο διακοπής αντίδρασης
	- Solucão de paragem	
SB 5x	- Tampone campione	- Sample buffer
	- Tampon Echantillons	- Tampón Muestras
	- Probenpuffer	- Ρυθμιστικό διάλυμα δειγμάτων
	- Diluente de amostra	