Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

Beta-2-Microglobulin ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAB2M1	Beta-2-Microglobulin ELISA	96 Tests

Intended Use:

Beta-2-Microglobulin (B2MG) ELISA is intended to be used for the quantitative determination of Beta-2-Microglobulin in human serum. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

Beta-2-Microglobulin is an 11.8K Dalton protein identical to the light chain of the HLA-A, -B and -C antigen. It is expressed on nucleated cells, and is found at low levels in the serum and urine of normal individuals. B2MG concentrations are elevated following inflammatory diseases, some viral diseases, renal dysfunction and autoimmune conditions. B2MG serum levels should be used in conjunction with other clinical data to assess the status of individuals with various clinical conditions including active rheumatoid arthritis and kidney disease.

The B2MG ELISA is based on a sandwich ELISA principle. The assay system utilizes one anti-B2MG antibody for solid phase immobilization and another monoclonal anti-B2MG antibody conjugated to horseradish peroxidase. Samples are incubated on the plate during which any B2MG in the sample interacts with the coated antibody and is bound to the plate surface. The wells are washed to remove excess sample then conjugate is added. The second, labelled antibody also binds to the B2MG, and after a wash step to remove unbound antibody, a solution of TMB is added which reacts with the label resulting in the development of a blue colour. The addition of acid stop solution stops the reaction, the colour is changed from blue to yellow and the absorbance measured at 450nm. The concentration of B2MG is directly proportional to the intensity of colour developed.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with monoclonal Anti-B2MG antibody. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable until expiry at 2-8°C.
B2MG Calibrators	6x0.5ml	6 vials containing B2MG at concentrations of 0.0, 0.5, 2, 5, 10 and 20 µg/ml made up in a human serum matrix. THE EXACT CONCENTRATIONS ARE PROVIDED ON THE VIAL LABEL. CONCENTRATIONS GIVEN IN THE IFU ARE SUBJECT TO CHANGE. Ready to use. Once opened store at 2-8°C.
Sample Diluent	1x100ml	1 vial containing diluent buffer. Once open stable until expiry at 2-8°C.
Enzyme Conjugate	1x22ml	1 vial containing 22ml of HRP labelled monoclonal Anti-B2MG antibody in buffered saline. Once open, stable until expiry at 2-8°C.
Wash Buffer Concentrate (50X)	1x15ml	PBS-Tween at pH 7.4. 50X concentrate. The concentrate must be diluted with 735ml of distilled water before use. Once diluted it is stable at room temperature for two months.
Substrate Solution	1x12ml	Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable until expiry at 2-8°C.
Stop Solution	1x12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable until expiry at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water, Vortex mixer, Micropipettes, Incubator, Microplate Reader and Microplate washer.

Specimen Collection:

Serum should be prepared from whole blood specimen obtained by acceptable medical techniques. The kit is for use with serum samples without additives only and not plasma since EDTA, heparin and oxalate can interfere with test procedures.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination. Place unused wells in zip-lock bag with desiccant provided and return to 2-8°C, under which conditions the wells will remain stable until the labelled expiry date. Seal and return all the other unused reagents to 2-8°C, under which conditions the stability will be retained until the labelled expiry date.

Procedure:

Reagent preparation:

1. Bring all reagents to room temperature (18-25°C) prior to use.
2. Dilute the wash buffer concentrate with 735ml of Distilled water (yielding a total volume of 750ml). Once diluted the wash solution is stable for 2 months at room temperature. Mix well before use.

STEP 1

Preparation: Remove the number of wells required and number each well for the assay series. Both the patient serum samples and control serum need to be diluted before use. In a series of tubes mix 10 µl serum in 1 ml Sample Diluent (101 fold dilution). Do not dilute the calibrators; they have already been prediluted.

STEP 2

Addition of Samples and Calibrators: Add 5 µl of calibrators, diluted samples and diluted controls to appropriate wells.

STEP 3

Addition of Sample Diluent: Add 200 µl Sample Diluent to each well. Mix for 10 seconds by gently tapping the side of the plate.

STEP 4

Incubation: Cover the plate with the plate cover and incubate at 37°C for 30 minutes.

STEP 5

Washing: At the end of the incubation period, remove the plate cover and discard the contents of the wells. Wash each well 5 times with 350 µl of diluted wash buffer. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of Enzyme Conjugate: Add 200 µl of the Enzyme Conjugate to each well. Shake the plate for 10 seconds to ensure that the added components are well mixed.

STEP 7

Incubation: Cover the plate with the plate cover and incubate at 37°C for 30 minutes.

STEP 8

Washing: At the end of the incubation period, remove the plate cover and discard the contents of the wells. Wash each well 5 times as described in Step 5. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of the Substrate: Add 100 µl of Substrate Solution to each well. Mix gently for 10 seconds.

STEP 10

Incubation: Cover the plate with the plate cover and incubate for 20 minutes at room temperature. Ensure that the incubation is done in the dark.

STEP 11

Stopping the Reaction: Add 100 µl of the Stop solution into each well. Shake the plate gently to mix until the solution changes completely from blue to yellow.

STEP 12

Measurement: Read the absorbance of the wells at 450/630nm using a microplate reader within 15 minutes of adding the Stop Solution. Record the absorbance for each well.

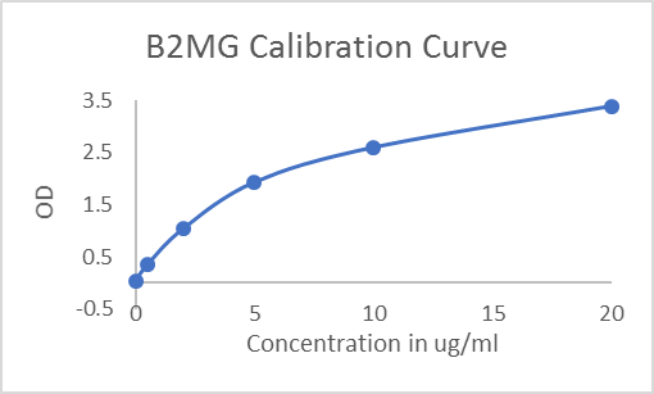
Note: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
All calibrators, samples and controls must be added to the microplate wells within 5 minutes. Adjust the size of the run if manual pipetting is used to ensure sample addition is performed within this time.

Calculation of results:

- Record the absorbances obtained from the microplate reader. Ensure that mean absorbances are calculated for duplicate measurements.
- Plot the absorbance on Y axis and Concentration in µg/ml on X axis.
- Draw a point to point curve through the plotted points on a linear graph paper.
- To determine the concentration of an unknown sample, locate the absorbance of the sample on the Y axis and find the intersecting point on the curve. Read the concentration from the X axis by dropping a line from the intersecting point of the absorbance on the curve.

Example:

ID	ABSORBANCE OF CALIBRATORS	CONCENTRATION OF CALIBRATORS
CAL A	0.040	0.0 µg/ml
CAL B	0.344	0.5 µg/ml
CAL C	1.035	2.0 µg/ml
CAL D	1.930	5.0 µg/ml
CAL E	2.599	10 µg/ml
CAL F	3.394	20 µg/ml



Expected Values:
Healthy individuals are expected to have B2MG levels less than 2.0 µg/ml. It is recommended that each laboratory establish its own normal ranges.

Performance Characteristics:

1. **Intra assay Precision:**

Panel	Data no.	Mean	SD	CV%
Level 1	20	1.05 µg/ml	0.091	8.65 %
Level 2	20	3.39 µg/ml	0.26	7.60 %
Level 3	20	8.37 µg/ml	0.36	4.29 %

2. **Inter assay Precision:**

Panel	Data no.	Mean	SD	CV%
Level 1	16	1.06 µg/ml	0.099	9.31 %
Level 2	16	3.48 µg/ml	0.31	8.94 %
Level 3	16	8.44 µg/ml	0.43	5.03 %

3. **Sensitivity:**

The minimum detectable concentration of B2MG by this assay was found to be 0.2 µg/ml.

Linearity:
Two patient sera were serially diluted with 0 ng/ml standard in a linearity study. The average recovery was 102.0%

Sample A			
Dilution	Expected	Observed	% Recovery
Sample undil	14.65 µg/ml	14.65 µg/ml	
2x	7.33 µg/ml	7.01 µg/ml	95.7 %
4x	3.66 µg/ml	3.64 µg/ml	99.4 %
8x	1.83 µg/ml	1.86 µg/ml	101.6 %
16x	0.92 µg/ml	0.96 µg/ml	104.8 %
Average Recovery: 100.4%			

Sample B			
Dilution	Expected	Observed	% Recovery
Sample undil	12.44 µg/ml	12.44 µg/ml	
2x	6.22 µg/ml	6.32 µg/ml	101.6 %
4x	3.11 µg/ml	3.28 µg/ml	105.5 %
8x	1.56 µg/ml	1.59 µg/ml	102.3 %
16x	0.78 µg/ml	0.82 µg/ml	105.5 %
Average Recovery: 103.7%			

Cross Reactivity:
The following materials were tested for cross reactivity and the results are as follows:

Antigens	Concentration	Equivalent B2MG	% Cross reactivity
Ferritin	10000 ng/ml	0.0 µg/ml	0.0 %
PSA	10000 ng/ml	0.0 µg/ml	0.0 %
AFP	10000 ng/ml	0.0 µg/ml	0.0 %
CEA	10000 ng/ml	0.0 µg/ml	0.0 %
Human IgG	20.0 g/l	0.0 µg/ml	0.0 %
Bilirubin	0.3 mg/ml	0.0 µg/ml	0.0 %
Triglycerides	20 mg/ml	0.0 µg/ml	0.0 %
Hemoglobin	5 mg/ml	0.0 µg/ml	0.0 %

No High dose hook effect was observed up to 1000 µg/ml.

References:

1. Berggard I and Beam AG. Isolation and properties of a low molecular weight β2-globulin occurring in human biological fluids. J Biol Chem, 1968; 243: 4095-4103.
2. Grey HM, Kubo RT, Colon SM, Poulik MMD, Cresswell P, Springer T, Turner M and Strominger JL. The small subunit of HL-A antigens is β2-microglobulin. J Exp Med, 1973; 138: 1608-1612.
3. Nakamuro K, Tanigaki N and Pressman D. Multiple common properties of human β2-microglobulin and the common portion fragment derived from HL-A antigen molecules. Proc Nal Acad Sci, 1973; 70: 2863-2865.
4. Evrin PE and Wibell L. The serum levels and urinary excretion of β2-microglobulin in apparently healthy subjects. Scand J Clin Lab Invest, 1972; 29: 69-74.
5. Crisp AN, Coughlan RJ, Mackintosh D, Clark B and Panayi GS. β2-microglobulin plasma levels reflect disease activity in rheumatoid arthritis. J Rheumatol, 1983; 10; 954-956.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

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Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100
www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

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