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HUMAN BETA 2-MICROGLOBULIN ELISA

Cat. No.: PL1010

Enzyme Immunoassay for the quantitative determination of Beta 2-microglobulin in human serum, plasma, and urine.

Urinary excretion of Beta 2-microglobulin (beta 2M) is a widely used test of renal proximal tubular function.[1] Proximal tubular dysfunction leads to an increased urinary concentration.[2]

The serum level of beta 2-microglobulin is determined by the glomerular filtration rate and the rate of synthesis. Increased production, with raised serum levels, is sometimes observed in malignancy-mainly at an advanced state.[2]

A strong correlation between lupus nephritis (LN), disease activity, and serum beta 2-microglobulin (b2MG) was observed. Serum b2MG level can be used as a valuable predictor for LN, clinical disease activity, and damage score.[3]

Principle of BETA 2-microglobulin ELISA

The microtiter plate is coated with the antibody specifically binding the Beta 2-microglobulin. The human serum, plasma or urine is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with HRP-labelled detection antibody. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of the Beta 2-microglobulin the specimen. The concentration of Beta 2-miroglobulin in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.

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- **Add 100 µL of Standards, diluted QCs and Samples to the wells**
- Incubate for 1 hour at 25 °C, shaking at 300 rpm
- **3-times wash the wells (350 µL/well)**
- **Add 100 µL of HRP-conjugated Antibody to the wells**
- Incubate for 1 hour at 25 °C, shaking at 300 rpm
- **3-times wash the wells (350 µL/well)**
- **Add 100 µL of Substrate Solution to the wells**
- Incubate for 10 min in the dark at 25 °C, NO shaking
- **Add 100 µL of Stop Solution to the wells**
- **Read the signal at 450 nm (450/630 nm) within 15 min**

Kit contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Antibody-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	2×13 mL
Wash Buffer 15× conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

Material required but not supplied

1. Glassware and test tubes.
2. Microtiter plate washer.
3. Precision pipettes (various volumes) with tips.
4. Orbital shaker.
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation.

Warnings and precautions

1. For research use only.
2. For professional laboratory use.
3. The reagents with different lot numbers should not be mixed.
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25 °C ±2 °C).

Storage conditions

1. The kit must be stored at 2–8 °C.
2. The opened components can be stored for one week at 2–8 °C.

Preparation of reagents

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25 °C ±2 °C.

Preparation of Standards

Reconstitute lyophilized Human Beta 2-microglobulin Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min prior to use. The concentration of human Beta 2-microglobulin in Master Standard is 40 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 40 ng/mL (lyophilized)	1000 µL	40 ng/mL
Std2	250 µL of Std1	250 µL	20 ng/mL
Std3	250 µL of Std2	250 µL	10 ng/mL
Std4	250 µL of Std3	250 µL	5 ng/mL
Std5	250 µL of Std4	250 µL	2.5 ng/mL
Std6	250 µL of Std5	250 µL	1.25 ng/mL
Blank		200 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:300 in Dilution Buffer, prior to use, see Preparation of samples.

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Preparation of Wash Buffer 1×

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15 × conc. to 700 mL of deionized /distilled water (dH_2O). Mix well. Store at 4 °C for two weeks or at -20 °C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70 °C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of serum and plasma is 1:300. It is recommended to use the two-step dilution. Dilution A (15×) for both singlets and duplicates: 5 µL of samples +70 µL of Dilution Buffer. Dilution B (20×): 10 µL of Dilution A +190 µL of Dilution Buffer, for singlets; 15 µL of Dilution A +285 µL of Dilution Buffer, for duplicates. Recommended dilution of urine is 1:10, i.e., 20 of samples +180 µL of Dilution Buffer, for singlets, 30 of samples +270 µL of Dilution Buffer, for duplicates.

Do not store the diluted samples.

Assay procedure

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for 2 hours at 25 °C ±2 °C, shaking at 300 rpm.
3. Wash the wells 3-times with 1× Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for 1 hour at 25 °C ±2 °C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL Substrate solution, incubate for 10 min at 25 °C ±2 °C. Avoid exposure to the light during this step.
7. Pipette 100 µL of STOP solution.
8. Read the signal at 450 or 450/630 nm within 10 min.

Performance characteristics

Samples used in the tests were diluted 1:300 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human Beta 2-microglobulin giving absorbance higher than absorbance of blank +3 standard deviations, is better than 0.42 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	1049	49	5
2	2178	131	6

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	1191	115	10
2	1469	88	6

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		2195	-	-
	2×	1048	1097	96
	4×	495	549	90
	8×	235	274	86
2		1295	-	-
	2×	586	647	91
	4×	324	324	100
	8×	138	162	85

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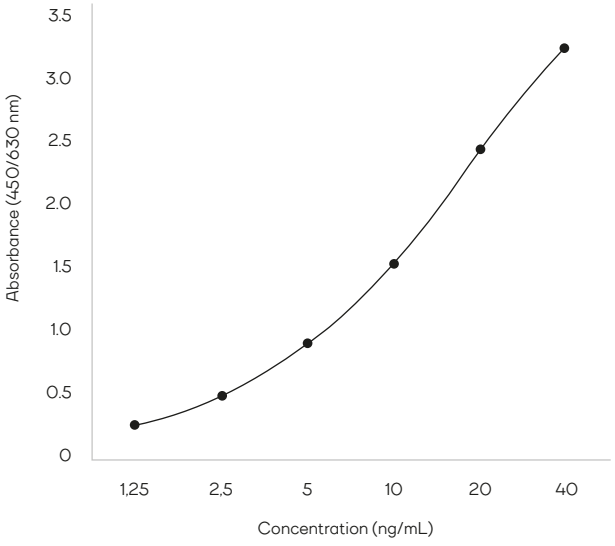
Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	1059	-	-
	3000	4186	4059	103
	1500	2937	2559	115
	750	1934	1809	107

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.

Human Beta 2-microglobulin standard curve



Resources

1 Davey PG, Gosling P. beta 2-Microglobulin instability in pathological urine. Clin Chem. 1982 Jun;28(6):1330-3. PMID: 6176371.

2 Karlsson FA, Wibell L, Evrin PE. beta 2-Microglobulin in clinical medicine. Scand J Clin Lab Invest Suppl. 1980;154:27-37. PMID: 6163193.

3 Gamal DM, Badr FM, Taha SIAEF, Moustafa NM, Teama MAEM. Serum beta-2 microglobulin as a predictor of nephritis, disease activity, and damage score in systemic lupus erythematosus: a cross-sectional study. Rheumatol Int. 2022 Oct 7. doi: 10.1007/s00296-022-05221-1. Epub ahead of print. PMID: 36205758.

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