Beta-2 Microglobulin (B2M) ELISA Kit

05/18

(Catalog # E4563-100, 100 assays; Store at 4°C)

I. Introduction:

Beta-2 Microglobulin (B2M) is a component of MHC class I molecules, which are present on all nucleated cells. In patients on long-term hemodialysis, Beta-2 Microglobulin can aggregate into amyloid fibers that deposit in joint spaces, a disease, known as dialysis-related amyloidosis. BioVision's Beta-2 Microglobulin sandwich ELISA Kit is designed to detect B2M in serum and urine. It can also be adapted for other fluids.

II. Application:

This ELISA kit is used for in vitro quantitative determination of Beta-2 Microglobulin in human samples.
Detection Range: 0.156 - 10 ng/ml

III. Sample Type:

Human serum, plasma and urine

IV. Kit Contents:

Components	E4563-100	Part No.
Micro ELISA Plate	12 strips x 8 wells	E4563-100-1
Standard Set (6 tubes)	1 ml x 6	E4563-100-2.x
Sample Diluent	100 ml	E4563-100-3
Enzyme Conjugate Reagent	22 ml	E4563-100-4
TMB Reagent	11 ml	E4563-100-5
Stop Solution	11 ml	E4563-100-6
Wash Buffer (20X)	25 ml	E4563-100-7

V. User Supplied Reagents and Equipment:

- Distilled or deionized water
- Adjustable Precision pipettes and disposable pipette tips
- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper

VI. Storage Conditions:

- Kit can be used within one year if stored properly at 4°C.
- Keep microwells sealed in a dry bag with desiccants.
- Avoid expose test reagents to heat, sun or strong light.

VII. Sample and Reagent Preparation:

- Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipidic or turbid samples.
- Typically, specimens should be capped and may be stored for up to 48 hour at 2-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for up to 6 months prior to assay. Thawed samples should be inverted several times to mix prior to testing.
- Collect urine samples and store at 2-8°C for up to 5 days or at -20C for longer periods. Urine samples are diluted 1:10 by adding 50 µl urine to 450 µl sample diluent. Use same assay procedure as for serum test.
- Wash buffer: Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

VIII. Assay Protocol:

Precaution:

- Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.

Assay Procedure:

** Bring all specimens and kit reagents to room temperature and gently mix 30 minutes before the assay.

1. Pipette 20 µl of **standards, controls and specimens** into selected well in duplicate. Dispense 200 µl of **Sample Diluent** into each well. Thoroughly mix for 30 seconds to ensure proper mixing.
2. Incubate for 30 minutes at 37°C.
3. Wash the wells 3 times with 300 µl of **1X Wash buffer** using either a suitable plate washer or wash bottle. Be careful not to cross contaminate wells. Remove all residual liquid droplets after the last wash.
4. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds and Incubate at 37°C for 30 minutes.
5. Wash again as step 3.
6. Add 100 µl of **TMB Reagent** to each well. Incubate for 20 minutes at room temperature, preferably in the dark.
7. Add 100 µl of **Stop Solution** to each well. Shake the plate gently to mix the solution.
8. Read O.D. at 450 nm using ELISA reader within 15 min.

FOR RESEARCH USE ONLY! Not to be used on humans.

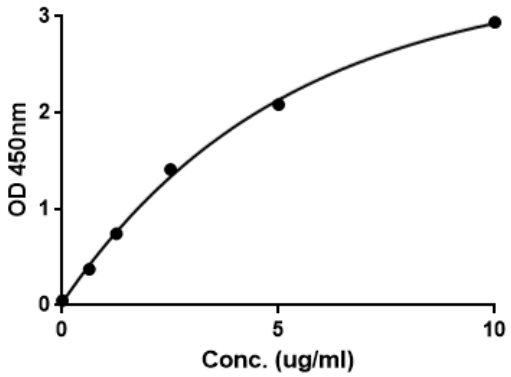
IX. Result Interpretation:

Calculation:

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of B2MG in µg/ml from the standard curve.
4. If the sample was diluted prior to the assay, multiply back the dilution factors to get actual sample concentration.

Example of a Standard Curve:

Standard	OD (450 nm)	Part No.
Standard 1 (0 µg/ml)	0.052	E4563-100-2.1
Standard 2 (0.625 µg/ml)	0.311	E4563-100-2.2
Standard 3 (1.25 µg/ml)	0.745	E4563-100-2.3
Standard 4 (2.5 µg/ml)	1.414	E4563-100-2.4
Standard 5 (5 µg/ml)	2.085	E4563-100-2.5
Standard 6 (10 µg/ml)	2.942	E4563-100-2.6



X. RELATED PRODUCTS:

- Anti-β2 Microglobulin Antibody (2F1) (Cat. No. A1025)
- Anti-Beta-2 Microglobulin Antibody (B2M/961) (Cat. No. A1440)

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