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Beta-2 Microglobulin ELISA

Catalog No. BM010T (96 Tests)

INTENDED USE

The Calbiotech, Inc. Beta 2 Microglobulin ELISA Kit is intended for the quantitative determination of Beta-2 Microglobulin (B2MG) Concentration in Human Serum. **For Research Use Only. For professional use only. Not for use in diagnostic procedures.**

SUMMARY AND EXPLANATION

β2-microglobulin is the light chain component of MHC class I molecules, found on all nucleated cells. Research uses include immunology, antigen presentation mechanisms, cell surface receptor studies, and renal physiology research.

PRINCIPLE OF THE TEST

The B2MG ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact β-2 Microglobulin molecule. Mouse monoclonal anti- B2MG antibody is used for solid phase immobilization (on the microtiter wells). A sheep anti-B2MG antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The diluted test sample is allowed to react first with the immobilized antibody for 30 minutes at 37°C. The sheep anti-B2MG-HRP conjugate is then added and reacted with the immobilized antigen for 30 minutes at 37°C, resulting in the B2MG molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, changing the color to yellow. The concentration of B2MG is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS PROVIDED		96 Tests
1.	Microwells coated Murine monoclonal anti-B2 MG antibody	12x8x1
2.	B2MG Reference Standards: 0, 0.625, 1.25, 2.5, 5, and 10	1 mL
3.	Sample Diluent, 100 ml.	100 mL
4.	Enzyme Conjugate Reagent, 22 ml	22 mL
5.	TMB Reagent (One-Step), 11 ml	11 mL
6.	Stop Solution (1N HCl), 11 ml.	11 mL

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

1. The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDELING

This assay is designed for use in compliance with applicable laws, regulations, and institutional policies. Handle and store samples using procedures appropriate for research use. Avoid repetitive freeze–thaw cycles.

1. 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.
2. Typicaly, 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.
3. Collect urine samples and store at 2-8°C for up to 5 days or at -20°C for longer periods. Urine samples are diluted 1:10 by adding 50 µl urine to 450µl sample diluent. Use same assay procedure as for serum test.

PREPERATION FOR ASSAY

1. All reagents should be brought to room temperature (20-25 °C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Reconstitute each lyophilized standard with 1.0 ml-distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

ASSAY PROCEDURE FOR SERUM AND PLASMA

1. Samples of patient serum, plasma and control serum need to be diluted before use for best results. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 10 µl serum with 1.0 ml Sample Diluent (101 fold dilution). Do not dilute the standards, they have already been pre-diluted 101 fold.
2. Secure the desired number of coated wells in the holder.
3. Dispense 20 µl of standards, diluted specimens, and diluted controls into appropriate wells.
4. Dispense 200 µl of Sample Diluent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix them completely.
6. Incubate at 37°C for 30 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300 µL of deionized water. Blot on absorbance paper or paper towel.
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
10. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
11. Incubate at 37°C for 30 minutes.
12. Remove the contents and wash the plate as described in step 7, 8, and 9.
13. Dispense 100 µl TMB Reagent into each well.
14. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 10 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

CALCULATION OF RESULTS FOR SERUM AND PLASMA

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and patient 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.

ASSAY PROCEDURE FOR URINE TEST

1. Urine Samples need 10-fold Dilution with the Sample Diluent (i.e. 50 µl urine + 450 µl Sample Diluentl).
2. Follow the same Assay Procedure for Serum/Plasma Test from step 2 to step 18.

CALCULATION OF RESULTS FOR URINE TEST

1. Calculate the mean absorbance value (A_{450}) for each reference standards, controls and patient 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.

Use the mean absorbance values for each specimen to determine the corresponding concentration of β2MG in µg/ml. Divide the calculated values by 10.1 (Since the β-2 Microglobulin standards have been prediluted 101 fold, the results obtained from urine samples should be further divided by 10.1). For instance, if the calculated value for a urine sample from the standard curve is 2.40 µg/ml; then the real value will be 2.40 µg/ml ÷ 10.1 = 0.238 µg/ml.3.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

Example of a Standard Curve

Results of a typical standard run with absorbency readings at 450 nm shown in the Y axis against B2MG concentrations shown in the X axis. This standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

B2MG (µg/ml)	Absorbance (450 nm)
0	0.052
0.625	0.377
1.25	0.745
2.5	1.414
5.0	2.085
10.0	2.942