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Beta-2 Microglobulin ELISA Kit

Name	Beta-2 Microglobulin ELISA Kit
INTENDED USE	The Beta 2 Microglobulin ELISA Kit is intended for the quantitative determination of Beta-2 Microglobulin (B2MG) Concentration in Human Serum.
SUMMARY AND EXPLANATION	Human -2 Microglobulin (B2MG) is an 11.8 kD protein identical to the light chain of the HLA-A, -B, and –C antigen. B2MG is expressed on nucleated cells, and is found at low levels in the serum and urine of normal individuals. B2MG concentrations are increased in inflammatory diseases, some viral diseases, renal dysfunction, and autoimmune diseases. A number of publications are available which explain the interpretation of B2MG serum levels in assessing the status of individuals with various clinical conditions.
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 -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 37C. The sheep anti-B2MG-HRP conjugate is then added and reacted with the immobilized antigen for 30 minutes at 37C, 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
Microwells coated Murine monoclonal anti-B2 MG antibody	12x8x1
B2MG Reference Standards: 0, 0.625, 1.25, 2.5, 5, and 10	1 ml
Sample Diluent, 100 ml.	100 ml
Enzyme Conjugate Reagent, 22 ml	22 ml
TMB Reagent (One-Step), 11 ml	11 ml
Stop Solution (1N HCl), 11 ml.	11 ml
Wash concentrate 20X: 1 bottle	25 ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

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:

The calibrator and controls 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, 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. 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. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
SPECIMEN COLLECTION AND HANDELING	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. Specimens should be capped and may be stored for up to 48 hour at 2-8C prior to assaying. Specimens held for a longer time can be frozen at -20C 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 2-8C for up to 5 days or at -20C for longer periods. Urine samples are diluted 1:10 by adding 50 l urine to 450l sample diluent. Use same assay procedure as for serum test.
REAGENT PREPARATION	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 (20-25C).
PREPERATION FOR ASSAY	All reagents should be brought to room temperature (20-25C) before use. All reagents should be mixed by

gently inverting or swirling prior to use. Do not induce foaming.

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 2-8C.

ASSAY PROCEDURE FOR SERUM AND PLASMA

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 l serum with 1.0 ml Sample Diluent (101 fold dilution). Do not dilute the standards, they have already been pre-diluted 101 fold.

Secure the desired number of coated wells in the holder.

Dispense 20 l of standards, diluted specimens, and diluted controls into appropriate wells.

Dispense 200 l of Sample Diluent into each well.

Thoroughly mix for 30 seconds. It is very important to mix them completely.

Incubate at 37C for 30 minutes.

Remove the incubation mixture by flicking plate contents into a waste container.

Remove liquid from all wells. Wash wells three times with 300 L of 1X wash buffer. Blot on absorbance paper or paper towel.

Strike the wells sharply onto absorbent paper or paper towels to remove all residual liquid droplets.

Dispense 200 l of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.

11. Incubate at 37C for 30 minutes.

Remove the contents and wash the plate as described in step 7, 8, and 9.

Dispense 100 l TMB Reagent into each well.

Gently mix for 10 seconds.

Incubate at room temperature in the dark for 20 minutes.

Stop the reaction by adding 100 l of Stop Solution to each well.

	Gently mix for 10 seconds. It is important to make sure that all the blue color changes to yellow color completely. Read absorbance at 450nm with a microtiter well reader within 15 minutes.
CALCULATION OF RESULTS FOR SERUM AND PLASMA	Calculate the mean absorbance value (A450) for each set of reference standards, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in g/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 B2MG in g/ml from the standard curve.
ASSAY PROCEDURE FOR URINE TEST	Urine Samples need 10 fold Dilution with the Sample Diluent (i.e. 50 l urine + 450 l Sample Diluentl). Follow the same Assay Procedure for Serum/Plasma Test from step 2 to step 18.
CALCULATION OF RESULTS FOR URINE TEST	Calculate the mean absorbance value (A450) for each reference standards, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in g/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 2MG in g/ml. Divide the calculated values by 10.1 (Since the -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 g/ml; then the real value will be 2.40 g/ml 10.1 = 0.238 g/ml.3.
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 (g/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

LIMITATIONS OF THE PROCEDURE

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.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

EXPECTED VALUES AND SENSITIVITY

Healthy individuals are expected to have B2MG serum or plasma values 0 - 2.0 µg/ml and urine values 0 – 0.3 g/ml.

The minimum detectable sensitivity is estimated to be 0.1 µg/ml.

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