Beta-2 Microglobulin (B2MG) ELISA Kit Protocol

(Cat. No.:EK-310-61)

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INTENDED USE

For the quantitative determination of Beta-2 Microglobulin (B2MG) concentration in human serum.. **FOR RESEARCH ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES**

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.

TABLE OF CONTENT

Introduction and Protocol Overview 4

Principle of the Test 4

Storage 5

List of Components 5

Specimen Collection and Preparation 6

Reagant Preparation 6

Assay Procedure 6

Calculation of Results 7

Example of Standard Curve 8

Expected Values and Sensitivity 9

References 9

INTRODUCTION AND PROTOCOL OVERVIEW

Human Beta-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 Beta-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.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

STORAGE

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement. DO NOT FREEZE

LIST OF COMPONENTS

Materials Provided with the Kit:

- Murine monoclonal anti-B2 MG antibody coated microtiter wells, 96 wells.
- B2MG Reference Standards: 0, 0.625, 1.25, 2.5, 5, and 10 µg/ml, 1 set, prediluted 101-fold, lyophilized.
- Sample Diluent, 100 ml.
- Enzyme Conjugate Reagent, 22 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided:

- Precision pipettes: 10 µl, 20 µl, 100 µl, 200 µl, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

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. Specimens should be capped and may be stored for up to 48 hour at 2-8℃ prior to assaying. Specimens held for a longer time can be frozen at -20℃ 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℃ for up to 5 days or at -20℃ 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.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°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℃.

ASSAY PROCEDURE

1. **Subject samples 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℃ for 30 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste

container.

8. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water 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.**

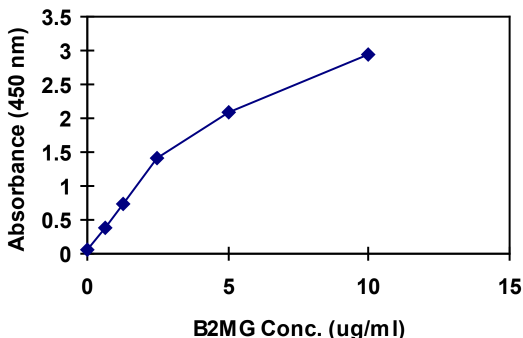
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A450) 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.

EXAMPLE OF 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



EXPECTED VALUES AND SENSITIVITY

Healthy individuals are expected to have B2MG values 0 - 2.0 µg/ml in serum. The minimum detectable sensitivity is estimated to be 0.1 µg/ml.

REFERENCES

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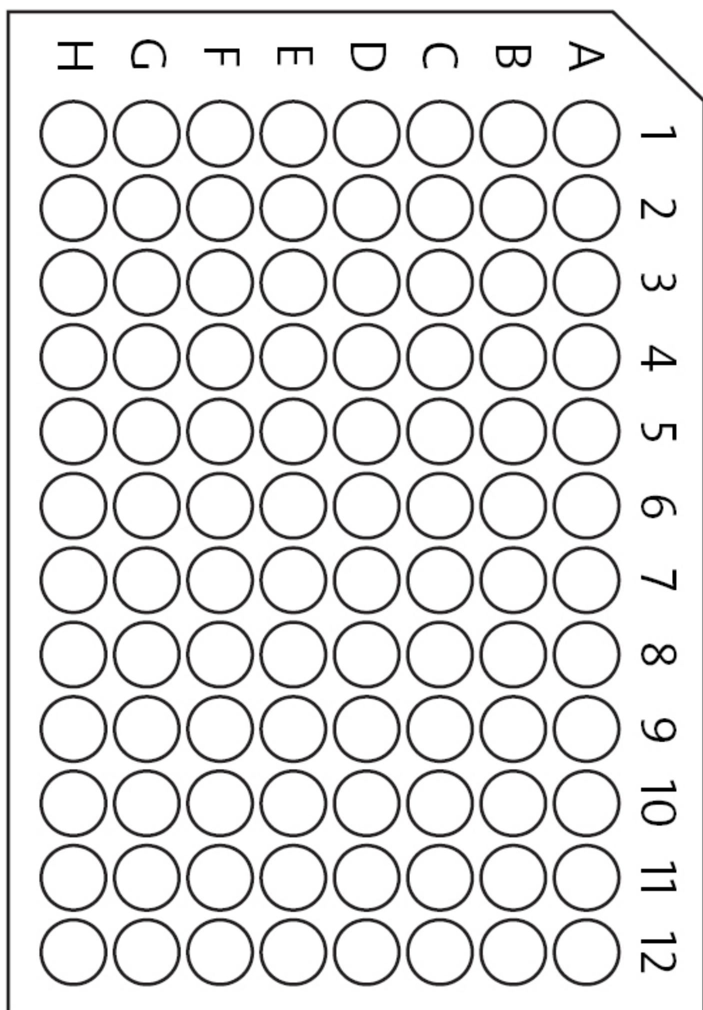
ASSAY DIAGRAM



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