BETA-2 MICROGLOBULIN ENZYME IMMUNOASSAY TEST KIT

Catalog Number: 10102

Enzyme Immunoassay for the Quantitative Measurement of Beta-2 Microglobulin (B2MG) in Human Serum

Introduction

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. The enzyme immunoassay allows the quantitative determination of B2MG from serum. In this assay, the B2MG in the samples is bound to an available excess of monoclonal antibodies against B2MG, which are immobilized to the surface of the microtiter wells. After a washing step to remove all foreign substances, the quantification of bound B2MG is carried out by adding an enzyme (horseradish peroxidase or HRPO) labeled antibody, which also binds to the B2MG. The amount of bound enzyme is directly proportional to the B2MG content. The substrate is then converted to a chromogenic compound, which can be determined photometrically at 450 nm.

Materials and Components

Materials provided with the test kit:

- Anti-Beta-2 MG antibody coated microtiter plate with 96 wells
- Sample diluent, 100mL
- Enzyme conjugate reagent, 22 mL
- B2MG reference standards, 1 set (liquid,ready to use) or lyophilized form.
- Wash Buffer Concentrate(50X),15mL
- TMB Substrate , 12mL
- Stop Solution, 12mL

Materials required but not provided:

- Precision pipettes and tips, 0.5~10 µL 0.04~0.2mL, 1.0~ 5mL.
- Distilled water.
- Disposable pipet tips.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.

Use of the B2MG EIA Test

The Beta-2 Microglobulin EIA Test is an enzyme immunoassay (EIA) for the measurement of Beta-2 Microglobulin in serum as an aid in the diagnosis of active rheumatoid arthritis and kidney disease.

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. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.

3. 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.

Storage of test kits and instrumentation

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 expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

- 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.
- Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.
- If reference standards are lyophilized, reconstitute each standard with 0.5 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

Assay Procedure

1. Both the samples of patient serum 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 10µL serum with 1.0 mL Sample Diluent (101 fold dilution). Do not dilute the standards; they have already been pre-diluted.

2. Secure the desired number of coated wells in the holder. Dispense **5**µL of B2MG standards, diluted specimens, and diluted controls into appropriate wells. Dispense **200** µL Sample Diluent. Gently mix for 10 seconds. Incubate at 37°C for 30 minutes.

3. Remove the incubation mixture by emptying the plate contents into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer(1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.

4. Dispense **200**µL of enzyme conjugate reagent into each well. Gently mix for 10 seconds Incubate at 37°C for 30 minutes. Remove the contents and wash the plate as described in step 3 above.

5. Dispense 100µL TMB substrate reagent into each well. Gently mix for 10 seconds. Incubate at room temperature in the dark for 20 minutes.

6. Stop the reaction by adding 100µL of Stop Solution to each well. Gently mix for 10 seconds. It is very important to make sure that the blue color changes to yellow color completely . Read optical density at 450nm with a microtiter plate reader within 15 minutes.

Important Note

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

2.It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

3.Duplication of all standards and specimens, although not required, is recommended.

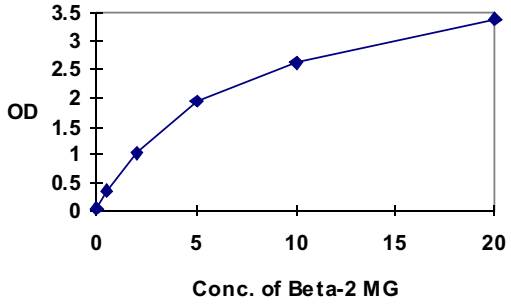
Calculation of Results

Calculate the mean absorbance value for each set of B2MG reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µg per mL on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. The best curve fit for the programming analysis is Quadratic. Use the mean absorbance values for each specimen to determine the corresponding concentration of B2MG in µg per mL from the standard curve. It is recommended that samples be analyzed in duplicates. Since the B2MG standards have already been diluted 101-fold, there is no need for the samples or controls to be multiplied by the dilution factor.

Example of Standard Curve

Results of typical standard run with optical density reading at 450nm 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 Values (µg/mL)	Absorbance (450 nm)
0	0.040
0.5	0.344
2.0	1.035
5.0	1.930
10.0	2.599
20.0	3.394



Expected Values and Sensitivity

Healthy individuals are expected to have B2MG values below 2.0 µg/mL.

Limitations of the Procedure

There are some limitation of the assay. We should let our customers know about that.

1)As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

2)Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee to eliminate all the effects of that.

3)The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

References

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