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 from serum. In this assay, the B2MG in the samples is bound

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

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, lipemic 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 until the expiration 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 should be used.

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 5µL 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 200µL 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

Absorbent paper or paper towel.
Microtiter plate reader.
Graph paper.

Calculation of Results

A standard curve is generated for each assay run using the control materials provided with the kit.

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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.

<table>
<thead>
<tr>
<th>B2MG Values (µg/mL)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.040</td>
</tr>
<tr>
<td>0.5</td>
<td>0.344</td>
</tr>
<tr>
<td>2.0</td>
<td>1.035</td>
</tr>
<tr>
<td>5.0</td>
<td>1.930</td>
</tr>
<tr>
<td>10.0</td>
<td>2.599</td>
</tr>
<tr>
<td>20.0</td>
<td>3.394</td>
</tr>
</tbody>
</table>

**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**


