INTENDED USE
For the direct quantitative determination of β₂-Microglobulin by an enzyme immunoassay in human serum.

PROCEDURE OF THE TEST
The procedure of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls, and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing of the substrate strip removes unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microplate reader. The intensity of the colour formed is inversely proportional to the concentration of the antigen in the sample. A set of standards is used to plot a standard curve on a microtiter plate reader. The intensity of the colour formed will show little variations in the normal level for β₂-M in males, pre- and postmenopausal females. The average tested was 92, showing little differences in the normal level for β₂-M.

CLINICAL APPLICATIONS
• Clinical Trends:
  1. All the reagents within the kit are calibrated for the direct determination of β₂-M in human serum. The kit is not calibrated for the detection of β₂-M in saliva, plasma or other specimens of human or animal origin.
  2. Do not use grossly hemolyzed, grossly lipemic, icteric or coagulated serum.
  3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false negative results.
  4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
  5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the presence of heterogeneous antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences during serological tests. Consequently, the clinical diagnosis should include all aspects of a patient’s background including the frequency of exposure to animals/products if false results are suspected.
  6. Some individuals may have antibodies to mouse protein which can possibly interfere with this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL
Human serum that may be used in the preparation of the controls and standards has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HIV and Human Immunodeficiency Virus type 2 (HIV-2) and found to be negative. No test method however, can offer complete assurance that HIV, HIV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential bio-hazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE
Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow to clot. Centrifuge and carefully remove the serum layer. Store for 24 hours at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT
This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED
1. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED
1. Mouse Anti-β₂-M Antibody-Coated Break-Apart Well Microplate – Ready To Use
   Contents: One 12-well (128) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
2. β₂-M-Horseradish Peroxidase (HRP) Conjugate
   Contents: β₂-M-HRP conjugate in a protein-based buffer with a non-mercuric preservative.
   Volume: 4 mL/bottle
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
3. β₂-M Calibrators – Ready To Use
   Contents: Six vials containing β₂-M in a protein-based buffer with a non-mercuric preservative. Prepared by spiking buffer with defined quantities of β₂-M.
   Volume: 500 μL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
4. Controls
   Contents: Two vials containing β₂-M in a protein-based buffer with a non-mercuric preservative. Prepared by spiking buffer with defined quantities of β₂-M. Prepared by spiking buffer with defined quantities of β₂-M.
   Volume: 0.5 mL/vial
   Storage: Refrigerate at 2–8°C
   Stability: 12 months in unopened vials or as indicated on label.
5. Wash Buffer Concentrate – Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 16 mL/bottle
   Stability: 12 months or as indicated on label.
6. Buffer – Ready To Use
   Contents: One bottle containing a protein-based buffer with a non-mercuric preservative.
   Volume: 15 mL/bottle
   Stability: 12 months or as indicated on label.
7. TMB Substrate – Ready To Use
   Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO buffer.
   Volume: 16 mL/bottle
   Stability: Refrigerate at 2–8°C
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.
8. Stopping Solution – Ready To Use
   Contents: One bottle containing 1M sulfuric acid.
   Volume: 6 mL/bottle
   Stability: Refrigerate at 2–8°C
   Storage: Refrigerate at 2–8°C
   Stability: 12 months or as indicated on label.

CALIBRATION AND QUALITY CONTROL

Calibrator Concentration Volume
Calibrator A 0 mg/L 2.0 mL
Calibrator B 0.2 mg/L 0.5 mL
Calibrator C 0.6 mg/L 0.5 mL
Calibrator D 1.0 mg/L 0.5 mL
Calibrator E 4 mg/L 0.5 mL
Calibrator F 10 mg/L 0.5 mL

Storage: Refrigerate at 2–8°C
Stability: 12 months in unopened vials or as indicated on label.

For Research Use Only. Not for Use in Diagnostic Procedures.
CALCULATIONS
1. Prepare working solutions of the β2-M-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 100 μL of the conjugate working solution into each well. (The use of a washer is recommended.)
4. Incubate on a plate shaker for 15–20 minutes at room temperature.
5. Pipette 50 μL of stopping solution into each well at timed intervals.
6. Wash the wells 3 times with 300 μL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
7. Pipette 150 μL of TMB substrate into each well at timed intervals.
8. Incubate for desired OD (450 nm) at room temperature. (If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.)
9. Pipette 300 μL of diluted wash buffer.

EXPECTED NORMAL VALUES
As for all clinical assays the laboratory should collect data and establish their own range of expected normal values.

INTRA-ASSAY PRECISION
Two patient serum samples were diluted with calibrator A and B. The results (in mg/L) are tabulated below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked</td>
<td>0.469</td>
<td>0.456</td>
<td>1.57</td>
</tr>
<tr>
<td>2 Unspiked</td>
<td>0.206</td>
<td>0.212</td>
<td>4</td>
</tr>
<tr>
<td>3 Unspiked</td>
<td>0.116</td>
<td>0.112</td>
<td>10</td>
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</table>

RECOVERY
Spike samples were prepared by adding defined amounts of β2-M to patient serum samples (1:1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15.96</td>
<td>15.4</td>
<td>98.4</td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>1.07</td>
<td>1.1</td>
<td>98.1</td>
</tr>
<tr>
<td>1</td>
<td>0.77</td>
<td>0.8</td>
<td>96.3</td>
</tr>
<tr>
<td>2</td>
<td>17.71</td>
<td>17.3</td>
<td>98.6</td>
</tr>
<tr>
<td>2</td>
<td>3.63</td>
<td>3.54</td>
<td>102.5</td>
</tr>
<tr>
<td>2</td>
<td>1.94</td>
<td>1.9</td>
<td>98.4</td>
</tr>
<tr>
<td>2</td>
<td>0.90</td>
<td>0.9</td>
<td>99.9</td>
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LINEARITY
Two patient serum samples were diluted with calibrator A.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Obs. Result</th>
<th>Exp. Result</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.542</td>
<td>2.558</td>
<td>0.9999</td>
</tr>
<tr>
<td>B</td>
<td>1.006</td>
<td>1.121</td>
<td>0.6</td>
</tr>
<tr>
<td>C</td>
<td>0.447</td>
<td>0.441</td>
<td>0.444</td>
</tr>
<tr>
<td>D</td>
<td>0.206</td>
<td>0.212</td>
<td>0.209</td>
</tr>
</tbody>
</table>

REFERENCES