

# Product Information

## Beta-2 Microglobulin ELISA

Catalog Number **SE120150**

Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

Human  $\beta_2$  Microglobulin (B2MG) is an 11.8 kDa 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 Beta-2 Microglobulin ELISA Kit is intended for the quantitative determination of  $\beta_2$  microglobulin (B2MG) concentration in human serum or urine. 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 multiwell plates. 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.

### Components

Materials Provided	96 Tests
Microwells coated murine monoclonal anti-B2MG antibody	12 x 8 x 1
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

### Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

#### Sample 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 °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.

3. Collect urine samples and store at 2–8 °C for up to 5 days or at –20 °C for longer periods. See separate Procedure for assay of urine samples.

#### B2MG Reference Standards

Reconstitute each lyophilized standard with 1.0 mL of 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 °C.

#### 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 (18–26 °C).

#### **Storage/Stability**

Store the kit at 2–8 °C.

#### **Procedure**

**Notes:** The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

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.

All reagents should be brought to room temperature (18–26 °C) before use. All reagents should be mixed by gently inverting or swirling prior to use. Do not induce foaming.

#### Assay Procedure for Serum and Plasma

1. Samples of 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 µL of serum with 1.0 mL of Sample Diluent (101-fold dilution). **Note: 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 °C for 30 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbent paper or paper towel.
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual liquid 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 of 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.

#### Assay Procedure for Urine

1. Urine samples need 10-fold Dilution with the Sample Diluent (*i.e.*, 50 µL of urine plus 450 µL of Sample Diluent).
2. Follow the Assay Procedure for Serum and Plasma from steps 2–18.

## Results

### Calculations of Results for Serum and Plasma

1. Calculate the mean absorbance value ( $A_{450}$ ) for each set of reference standards, controls, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  from the standard curve.

### Calculations of Results for Urine

1. Calculate the mean absorbance value ( $A_{450}$ ) for each reference standards, controls, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$ . Divide the calculated values by 10.1 (Since the  $\beta_2$  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  $\mu\text{g/mL}$ ; then the real value will be  $2.40 \mu\text{g/mL} \div 10.1 = 0.238 \mu\text{g/mL}$ .

**Notes:** Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the 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.

### Example of a Standard Curve

Results of a typical standard run with absorbence readings at 450 nm. 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 ( $\mu\text{g/mL}$ )	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

### Expected values and sensitivity

Healthy individuals are expected to have B2MG serum or plasma values of 0–2.0  $\mu\text{g/mL}$  and urine values of 0–0.3  $\mu\text{g/mL}$ . The minimum detectable sensitivity is estimated to be 0.1  $\mu\text{g/mL}$ .

### References

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2. Grey, H.M. et al., 1973. The small subunit of HL-A antigens is  $\beta$ 2-microglobulin. *J. Exp. Med.*, 138: 1608-1612.
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