



## DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F,  
Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

[onestep@rapidtest.com](mailto:onestep@rapidtest.com)

[technicalsupport@rapidtest.com](mailto:technicalsupport@rapidtest.com)

[www.rapidtest.com](http://www.rapidtest.com)

IVD



See external label



2°C-8°C



Σ=96 tests

REF

Cat # 9076-16

**CHEMILUMINESCENCE**  
**ENZYME IMMUNOASSAY (CLIA)**  
**BETA-2 MICROGLOBULIN (B2MG)**

# B2-Microglobulin

**Cat # 9076-16**

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

## INTRODUCTION OF CHEMILUMINESCENCE IMMUNOASSAY

Chemiluminescence Immunoassay (CLIA) detection using Microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have

been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

The CLIA Kits have been validated on the **MPL2** microplate luminometer from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

## INTRODUCTION OF B2 MG IMMUNOASSAY

Beta-2-microglobulin ( $\beta$ 2-MG) is expressed by the nucleated cells of the body and on many tumor lines. Human  $\beta$ 2-MG is a low molecular weight protein (MW 11600) consisting of a single polypeptide chain of 99 amino acids. It is identical to the small chain of the HLA-A, -B, and -C major histocompatibility complex antigens. In structure and amino acid sequence, it resembles the CH3 region of IgG, though it is antigenically distinct.

$\beta$ 2-MG is eliminated via the kidneys. After filtration through the glomeruli, it is reabsorbed and catabolized by the proximal tubular cells through endocytosis. It is found at low levels in the serum and

urine of normal individuals. Typically only trace amounts of  $\beta$ 2-MG

are excreted in the urine and higher rates are interpreted as evidence of tubular dysfunction. Urinary excretion is markedly increased in tubulointerstitial disorders, and where aminoglycosides and anti-inflammatory compounds are present.  $\beta$ 2-MG is also excreted in increased amounts in the urine of patients with upper urinary tract infections<sup>10</sup> and connective-tissue diseases such as rheumatoid arthritis and Sjogren's syndrome.

Elevated serum concentrations in the presence of normal glomerular filtration rate suggest increased  $\beta$ 2-MG production or release. In patients with rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis and some viral diseases including cytomegalovirus, non-A and non-B hepatitis and infectious mononucleosis, the  $\beta$ 2-MG serum level changes in relation to disease activity.

## TEST PRINCIPLE

The B2 MG EIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The B2 MG molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody-enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by adding the substrate reagents and undergoing the chemiluminescent reactions. The intensity of the emitting light from the associated well is proportional to the amount of enzyme present and is directly related to the amount of B2 MG antigen in the sample. By reference to a series of B2 MG standards assayed in the same way, the concentration of B2 MG in the unknown sample is quantified.

## MATERIALS AND COMPONENTS

### **Materials provided with the test kits:**

1. Anti-B2 MG antibody coated 96 well microtiter plate.
2. Sample Diluent, 100 ml.
3. Enzyme conjugate reagent, 22 ml.
4. B2 MG reference standards, containing 0, 0.5, 2.0, 5.0, 10 and 20  $\mu$ g/ml B2 MG, 1:100 prediluted liquid, ready for use.
5. 50x Wash Buffer Concentrate, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml
7. Chemiluminescence Reagent B, 6.0 ml

### **Materials required but not provided:**

1. Distilled water.
2. Precision pipettes: 0.5~10 $\mu$ l, 0.05~ 0.2ml, 1.0ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Reagent A and B.
5. Microtiter well luminometer.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

## REAGENT PREPARATION

1. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

## ASSAY PROCEDURE

### **Important Note:**

**The B2 MG standards have already been prediluted and are ready for use. Please DO NOT dilute again!**

1. Patient serum and control serum should be diluted, 101 fold, before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 10  $\mu$ l serum with 1.0 ml Sample Diluent.
2. Secure the desired number of coated wells in the holder. Dispense 5 $\mu$ l of B2MG standards, diluted specimens, and diluted controls into appropriate wells. Dispense 200  $\mu$ l Sample Diluent. Gently mix for 20 seconds.
3. Incubate at 37°C for 30 min.
4. Remove the incubation mixture by emptying the plate content into a waste container.
5. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
6. Strike the wells sharply onto absorbent paper to remove residual water droplets.
7. Dispense 200  $\mu$ l of enzyme conjugate reagent into each well. Gently mix for 10 seconds.
8. Incubate at 37°C for 30 min.
9. Remove the contents and wash the plate as described in step 4, 5 and 6 above.
10. Dispense 100 $\mu$ l Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
11. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

### **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

1. Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.
2. We recommend using proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available,

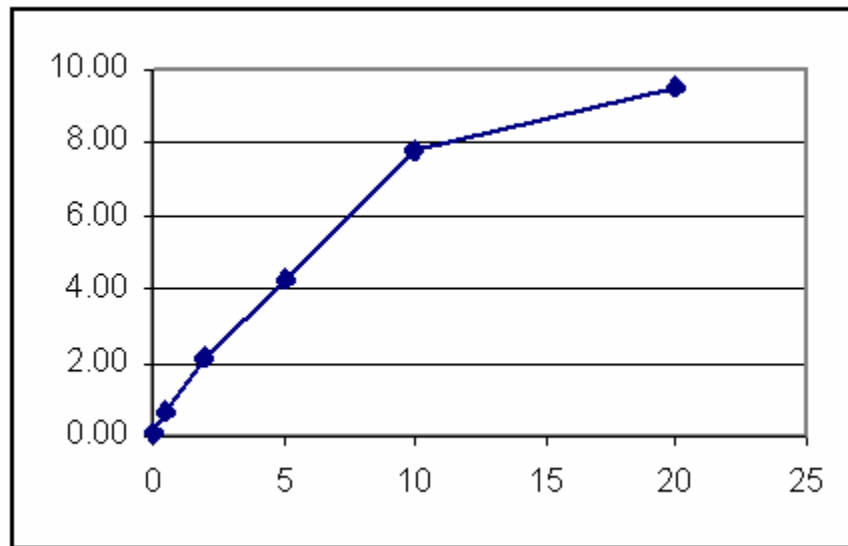
construct a standard curve by plotting the mean RLU obtained for each reference standard against B2 MG concentration in  $\mu\text{g/ml}$  on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.

- Using the mean absorbance value for each sample, determine the corresponding concentration of B2 MG in  $\mu\text{g/ml}$  from the standard curve.

### EXAMPLE OF STANDARD CURVE

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

B2 MG ( $\mu\text{g/ml}$ )	Relative Light Units (RLU) ( $10^5$ )
0	0.05
0.5	0.63
2	2.13
5	4.24
10	7.77
20	9.52



### EXPECTED VALUES AND SENSITIVITY

Healthy individuals are expected to have B2MG values below 2.0  $\mu\text{g/mL}$ .

### REFERENCES

- Berggard I and Beam AG: 1968. Isolation and properties of a low molecular weight  $\beta_2$ -globulin occurring in human biological fluids. *J Biol Chem* 243: 4095-4103.
- Grey HM, Kubo RT, Colon SM, Poulik MD, Cresswell P, Springer T, Turner M and Strominger JL: 1973. The small subunit of HL-A antigens is  $\beta_2$ -microglobulin. *J Exp Med* 138: 1608-1612.

3. Nakamuro K, Tanigaki N and Pressman D: 1973. Multiple common properties of human  $\beta$ 2-icroglobulin and the common portion fragment derived from HL-A antigen molecules. Proc Natl Acad Sci 70: 2863-2865.
4. Evrin PE and Wibell L: 1972. The serum levels and urinary excretion of  $\beta$ 2-microglobulin in apparently healthy subjects. Scand J Clin Lab Invest 29: 69-74.
5. Crisp AJ, Coughlan RJ, Mackintosh D, Clark B and Panayi, GS: 1983.  $\beta$ 2-microglobulin plasma levels reflect disease activity in rheumatoid arthritis. J Rheumatol 10: 954-956.

<b>Date Adopted :</b>	<b>Reference No.</b>
<b>2007-07-21</b>	<b>DA-B2-Microglobulin-2009</b>



**DIAGNOSTIC AUTOMATION, INC.**

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

**ISO 13485-2003**



Revision Date: 02/16/09