

# Human Beta2-Microglobulin ELISA Kit

For the Quantitative Determination of Human Beta2-Microglobulin  
(B2MG) Concentrations

Catalogue Number: EL10051

*96 tests*

FOR LABORATORY RESEARCH USE ONLY  
NOT FOR USE IN DIAGNOSTIC PROCEDURES



***ANOGEN***

2355 Derry Road East, Unit 23  
Mississauga, Ontario  
CANADA L5S 1V6  
Tel: (905) 677-9221 or (877) 755-8324  
Fax: (905) 677-0023

Email: [info@anogen.ca](mailto:info@anogen.ca) ♦ Web Site: [www.anogen.ca](http://www.anogen.ca)

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## INTENDED USE

This Human Beta2-Microglobulin ELISA Kit is to be used for *in vitro* quantitative determination of Beta2-Microglobulin (B2MG) concentrations in human serum, other biological samples, and cell culture supernatant. **The kit is intended for RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.**

## INTRODUCTION

Beta 2 microglobulin (B2MG) is initially discovered as a small serum protein of 12,000 Dalton. The small size allows B2MG to pass through the glomerular membrane, but it is normally completely reabsorbed in the proximal tubules. B2MG normally presents in minute amount, but increases its expressions, or adapts to the fibrillary configuration in certain pathological states.

B2MG is found to be a subunit of class I major histocompatibility complex (MHC I) that is expressed on all nucleated cell excluding red blood cells. B2MG non-covalently links with the  $\alpha 3$  domain of human leukocyte antigen (HLA) to form MHC I. Inside cytoplasm, MHC I binds to intracellular degraded foreign peptides, or self-peptides in some conditions, through the  $\alpha 1$  and  $\alpha 2$  variable domains of HLA. Subsequently, MHC I is anchored to the cell surface through the  $\alpha 3$  domain of HLA. By way of this procedure, MHC I presents the engulfed foreign peptides to (CD8+) cytotoxic T-cell, thus, plays an important role in cell-mediated immunity.

When binding with HFE protein, B2MG was found to increase iron uptake. B2MG is also associated with MHC I - like molecules such as CD1 and Qa. This immunoassay is a tool for investigation of the function and regulation of the protein.

## PRINCIPLE OF THE ASSAY

This B2MG enzyme linked immunosorbent assay (ELISA) kit uses a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for B2MG. Standards or samples are added to the microtiter plate wells and B2MG, if present, will bind to the antibody pre-coated on the wells. A preparation of horseradish peroxidase (HRP)-conjugated B2MG antibody is added to each well. The conjugated antibody will bind to the B2MG immobilized on the plate after incubation. All unbound components will be removed by subsequent washing procedure. Next, 3, 3', 5, 5' tetramethyl-benzidine(TMB), a substrate for HRP is added to each well. The colorimetric reaction is proportional to the B2MG content in each well. The reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at the wavelength of  $450\text{nm} \pm 2\text{nm}$ .

In order to quantify the amount of B2MG present in the sample, this Human B2MG ELISA Kit includes a set of calibration standards (6 standards). The calibration standards should be assayed at the same time as the samples to allow the operator to produce a standard curve of Optical Density (O.D.) versus B2MG concentration ( $\mu\text{g/mL}$ ). The concentration of B2MG in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

	<b>96 tests</b>
1. <b>B2MG MICROTITER PLATE</b> (Part EL51-1)_____	<b>96 wells</b>
Microtiter plate pre-coated with anti-human B2MG monoclonal antibody.	
2. <b>B2MG CONJUGATE</b> (Part EL51-2)_____	<b>12 mL</b>
Horseradish peroxidase conjugated anti-human B2MG antibody, with preservative. <i>Ready-to-use.</i>	
3. <b>B2MG STANDARD - 12µg/mL</b> (Part EL51-3)_____	<b>1 vial</b>
Lyophilized human B2MG in a buffered protein matrix, pre-diluted 101 fold.	
4. <b>B2MG STANDARD - 6µg/mL</b> (Part EL51-4)_____	<b>1 vial</b>
Lyophilized human B2MG in a buffered protein matrix, pre-diluted 101 fold.	
5. <b>B2MG STANDARD - 3µg/mL</b> (Part EL51-5)_____	<b>1 vial</b>
Lyophilized human B2MG in a buffered protein matrix, pre-diluted 101 fold.	
6. <b>B2MG STANDARD – 1.5µg/mL</b> (Part EL51-6)_____	<b>1 vial</b>
Lyophilized human B2MG in a buffered protein matrix, pre-diluted 101 fold.	
7. <b>B2MG STANDARD – 0.75µg/mL</b> (Part EL51-7)_____	<b>1 vial</b>
Lyophilized human B2MG in a buffered protein matrix, pre-diluted 101 fold.	
8. <b>B2MG STANDARD - 0µg/mL</b> (Part EL51-8)_____	<b>1 vial</b>
Lyophilized human B2MG in a buffered protein matrix, pre-diluted 101 fold.	
9. <b>SUBSTRATE A</b> (Part EL51-9)_____	<b>10 mL</b>
Buffered solution with H <sub>2</sub> O <sub>2</sub> .	
10. <b>SUBSTRATE B</b> (Part 30007)_____	<b>10 mL</b>
Buffered solution with TMB.	
11. <b>STOP SOLUTION</b> (Part 30008)_____	<b>10 mL</b>
2N Sulphuric Acid (H <sub>2</sub> SO <sub>4</sub> ). Caution: Caustic Material!	
12. <b>SAMPLE DILUENT</b> (Part EL51-10)_____	<b>60 mL</b>
Buffered protein matrix with preservative	

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100  $\mu\text{L}$  and 50-200  $\mu\text{L}$  for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Incubator ( $37\pm 2^\circ\text{C}$ ).
8. Microtiter plate reader ( $450\text{ nm}\pm 2\text{ nm}$ ).
9. Automatic microtiter plate washer or squirt bottle.
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water.
12. Plastic plate cover.
13. Disposable gloves.
14. Absorbent paper.
15.  $37^\circ\text{C}$  incubator.

## PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature ( $22\text{-}25^\circ\text{C}$ ) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at  $2\text{-}8^\circ\text{C}$  in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.  
Solid Wastes: Autoclave for 60 minutes at  $121^\circ\text{C}$ .  
Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat or flame.

## SAMPLE PREPARATION

### 1. COLLECTION, HANDLING AND STORAGE

Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) followed by serum extraction.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

### 2. SAMPLE DILUTION

Before assay is started, add 10 µl sample to 1ml B2MG Sample Diluent and mix well. The diluted samples should not be stored since they are not as stable as original samples. The lyophilized standards have been pre-diluted 101 fold and should be used without dilution after reconstitution.

## PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (22-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

**B2MG Standards:** Reconstitute each B2MG Standard vial with **0.6 ml** of distilled or de-ionized water. Allow each solution to sit for at least 15 minutes with gentle agitation. **The lyophilized standards have been pre-diluted 101 fold and should be used without dilution after reconstitution.** The reconstituted B2MG standards can be aliquoted to store at -20°C for at least 1 month. Avoid freeze-thaw cycles.

**B2MG Sample Dilution:** Before assay is started, add 5µl sample to 0.5ml B2MG Sample Diluent and mix well. The diluted samples should not be stored as they are not as stable as original samples.

**Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80wells)	6.0	6.0	12.0
12 strips (96wells)	7.0	7.0	14.0

## ASSAY PROCEDURE

1. Prepare all B2MG Standards and dilute samples with B2MG Sample Diluent before starting assay procedure (see Preparation of Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*
2. Secure a desired number of strips from the coated microtiter plate to the holder and add 100  $\mu$ L of Sample Diluent to each well. Add 10  $\mu$ L Standards or Samples to the appropriate wells. **IMPORTANT: COMPLETE MIXING SHOULD BE ACHIEVED BEFORE PROCEEDING.** Cover and incubate for **30 minutes at 37°C**.
3. Wash the Microtiter Plate using one of the specified methods described below:
 

Manual Washing: Remove the incubation mixture by aspirating the contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with de-ionized or distilled water and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate and blot dry by hitting the plate onto absorbent paper or paper towel until no moisture is visible. *Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain in the frame.*

Automated Washing: Aspirate all wells and wash plates **FIVE times** using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350  $\mu$ L/well/wash (range: 350-400  $\mu$ L). After final wash, invert plate and blot dry by hitting the plate onto absorbent paper or paper towel until no moisture is visible. *It is recommended that the washer be set for soaking time of 10 seconds or shaking time of 5 seconds between washes.*
4. Add 100  $\mu$ L of Conjugate into each well. Cover and incubate for **30 minutes at 37°C**
5. Repeat wash procedure as described in Step 3.
6. Add 100  $\mu$ L freshly mixed Substrate Solution to each well. Cover and incubate for **10-15 minutes at 37°C**.

7. Add 100  $\mu\text{L}$  of Stop Solution to each well. Mix well.
8. Read the Optical Density (O.D.) at 450nm using a microtiter plate reader within 10 minutes.

### CALCULATION OF RESULTS

A standard curve should be generated for each run. The standard curve is used to determine the amount of B2MG in an unknown sample. The standard curve is generated by plotting the average O.D. (450nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding B2MG concentration ( $\mu\text{g/mL}$ ) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard ( $0\mu\text{g/mL}$ ) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of B2MG in each sample, locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding B2MG concentration. If samples generate values greater than the highest standard, dilute the samples with the Sample Diluent and repeat the assay. The concentration read from the standard curve must be multiplied by the dilution factor.

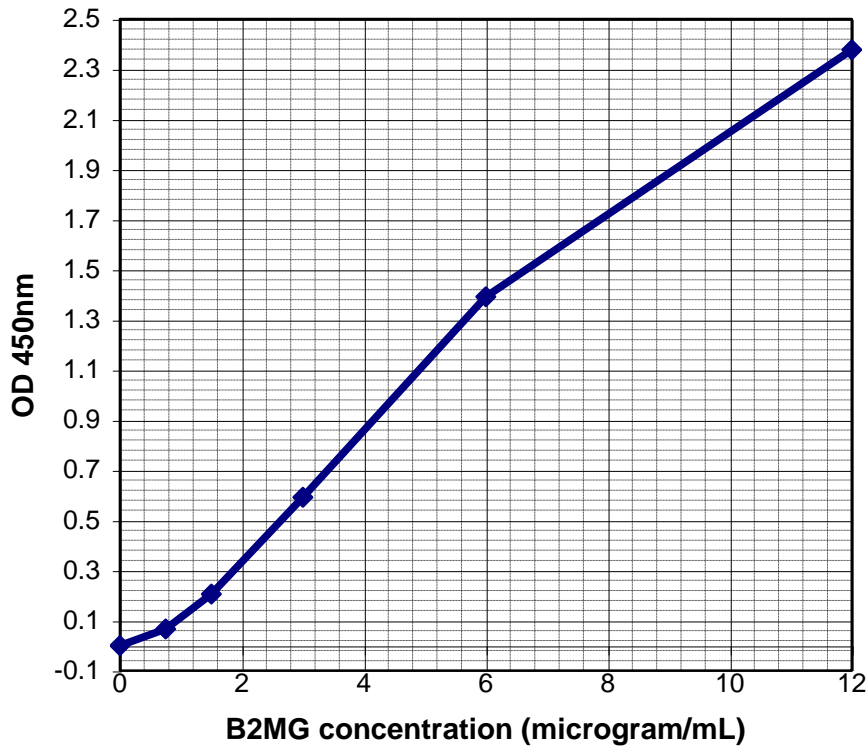
### TYPICAL DATA

Results of a typical standard run of B2MG ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variations in the results. The following examples are for the purpose of illustration only. To calculate the results of samples, a standard curve must run using the same lot of reagents

#### EXAMPLE

Standard ( $\mu\text{g/mL}$ )	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.047, 0.049	0.042	0.000
0.75	0.111, 0.106	0.108	0.066
1.5	0.245, 0.252	0.248	0.206
3	0.627, 0.640	0.633	0.591
6	1.393, 1.476	1.434	1.392
12	2.393, 2.446	2.419	2.377





## PERFORMANCE CHARACTERISTICS

### 1. PRECISION

Within-run (Intra-assay) coefficients of variation (CV) were determined by 16 replicate tests of two serum samples in one assay.

Samples	Mean Concentration ( $\mu\text{g/mL}$ )	Coefficients of Variation (CV)
1	0.526	7.43%
2	2.472	5.19%

Between-run (Inter-assay) coefficients of variation (CV) were determined by testing 2 serum samples in 6 different assays.

Samples	Mean Concentration ( $\mu\text{g/mL}$ )	Coefficients of Variation (CV)
1	0.536	8.04%
2	2.777	8.05%

### 2. SENSITIVITY

The minimal detectable dose of this B2MG assay was calculated by adding two standard deviations to the mean optical density value of 16 zero standard replicates and determining

the corresponding concentration from the standard curve. The minimal detectable dose for this human B2MG assay generated by this method is 0.2µg/mL.

### 3. SPECIFICITY

Cross-reaction with the following human serum components was not found.

	Concentration	Mean OD	Detected Value
CRP	2.61mg/mL	0.0415	Under detection limit
CEA	0.2mg/ML	0.0405	Under detection limit
PSA	0.1mg/mL	0.0420	Under detection limit
Human IgG	20mg/ML	0.0445	Under detection limit
Human serum albumin	100mg/ML	0.0360	Under detection limit

### 4. RECOVERY

The recovery rate of B2MG added to human sera ranged from 94% to 105%. The recovery rate of B2MG added to human urine samples ranged from 92% to 112%.

### 5. HOOK EFFECT

No hook effect was observed in this assay.

### 6. CALIBRATION

This immunoassay is calibrated against NIBSC/WHO B2MG First International Standard (Code 96/668).

**LIMITATIONS OF THE PROCEDURE: The assay is for research use only.**

## REFERENCES

1. Cunningham BA, Wang JL, Berggard I, Peterson PA. The complete amino acid sequence of B2M. *Biochem* 1973;12: 4811-22.
2. Gattoni-Celli S, Kirsch K, Timpane R, Isselbacher KJ. Beta 2-microglobulin gene is mutated in a human colon cancer cell line (HCT) deficient in the expression of HLA class I antigens on the cell surface". *Cancer Res.* 1992; **52** (5): 1201-4.
3. Grey HM, Kubo RT, Colon SM, Poulik MD, Cresswell P, Springer T, Turner M, Strominger JL. The small subunit of HLA antigens is B2M. *J Exp Med* 1973; 138:1608-12.
4. Saper MA, Bjorkman PJ, Wiley DC. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J. Mol. Biol.* 1991; (2): 277-319