β2-MICROGLOBULIN ENZYME IMMUNOASSAY TEST KIT
Catalog Number: EA100995

Enzyme Immunoassay for the Quantitative Determination of Beta-2-Microglobulin (β2-MG) Concentration in Human Serum

FOR IN VITRO DIAGNOSTIC USE

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

OriGene β2-Microglobulin Enzyme Immunoassay

INTENDED USE

The OriGene β2-Microglobulin ELISA is intended for the quantitative determination of β2-microglobulin in human serum. This assay is for use as an aid in the diagnosis of kidney disease.

INTRODUCTION

Beta-2-microglobulin (β2-MG) is expressed by the nucleated cells of the body and on many tumor lines. Human β2-MG is a low molecular weight protein (MW 11600) consisting of a single polypeptide chain of 99 amino acids.[1,2] It is identical to the small chain of the HLA-A, -B, and -C major histocompatibility complex antigens.[3-5] In structure and amino acid sequence, it resembles the CH3 region of IgG, though it is antigenically distinct.

β2-MG is eliminated via the kidneys. After filtration through the glomeruli, it is reabsorbed and catabolized by the proximal tubular cells through endocytosis.[6] It is found at low levels in the serum and urine of normal individuals. Typically only trace amounts of β2-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.[7-9] β2-MG is also excreted in increased amounts in the urine of patients with upper urinary tract infections[10] and connective-tissue diseases such as rheumatoid arthritis and Sjogren's syndrome.[11]

Elevated serum concentrations in the presence of normal glomerular filtration rate suggest increased β2-MG production or release. In patients with rheumatoid arthritis[12], systemic lupus erythematosus[13], sarcoidosis[14] and some viral diseases including cytomegalovirus, non-A and non-B hepatitis and infectious mononucleosis,[15,16] the β2-MG serum level changes in relation to disease activity.

The OriGene β2-MG ELISA provides a sensitive and reliable assay for the measurement of β2-microglobulin in human serum. The kit features a standard range of 0.625 to 10 µg/ml and will determine a minimum detectable concentration of 0.1 µg/ml. The assay provides results in less than 2 hours in a microtiter plate format.

PRINCIPLE OF THE ASSAY

The OriGene β2-MG ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes a monoclonal anti-β2-MG antibody for solid phase immobilization (on the microtiter wells). A sheep anti-β2-MG 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-β2-MG-HRPO conjugate is then added and reacted with the immobilized antigen for 30 minutes at 37°C, resulting in the β2-MG 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 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 1N HCl, changing the color to yellow. The concentration of β2-MG is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with murine monoclonal anti-β-2MG.
2. *Enzyme Conjugate Reagent (22 ml)*
   Contains sheep anti-β2-MG conjugated to horseradish peroxidase.
3. *Reference Standard Set (1 ml/vial)*
   Contains 0, 0.625, 1.25, 2.5, 5, and 10 µg/ml β2-MG in sample diluent, pre-diluted 101-fold, lyophilized.
4. *Sample Diluent (1 bottle, 100 ml)*
   2% BSA in buffered solution containing preservative.
5. *TMB Reagent (1 bottle, 11 ml)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
6. *Stop Solution (1N HCl) (1 bottle, 11 ml)*
   Contains diluted hydrochloric acid.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 5 µl, 10 µl, 50 µl, 100 µl, 200 µl, and 1.0 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm.
5. Vortex mixer, or equivalent
6. Absorbent paper
7. Graph paper
8. Optional quality control material (from OriGene or BioRad)

WARNINGS AND PRECAUTIONS

1. CAUTION**:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

SPECIMEN COLLECTION AND PREPARATION

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards should be stored sealed at 2-8°C, and are stable for at least 30 days at this temperature.

ASSAY PROCEDURE

NOTE: Patient samples and control serum need to be diluted before use. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 10 µl serum with 1.0 ml Sample Diluent (101 fold dilution). Do not dilute the standards, they have already been pre-diluted 101 fold.

1. Secure the desired number of coated wells in the holder.
2. Dispense 20 µl of standards, diluted specimens, and diluted controls into appropriate wells.
3. Dispense 200 µl of Sample Diluent into each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
4. Incubate at 37°C for 30 minutes.
5. Remove the incubation mixture by flicking plate contents into a waste container.
6. Rinse and flick the microtiter wells 5 times with deionized or distilled water. DO NOT USE TAP WATER.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
8. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
9. Incubate at 37 °C for 30 minutes.
10. Remove the contents and wash plate as described above in steps 5-7.
11. Dispense 100 µl TMB Reagent into each well. Gently mix for 10 seconds.
12. Incubate at room temperature, in the dark, for 20 minutes.
13. Stop the reaction by adding 100 µl of Stop Solution (1N HCl) to each well.
14. Gently mix for 10 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
15. Read absorbance at 450 nm with a microtiter well reader ***within 15 minutes.***

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µg/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of β2-MG in µg/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

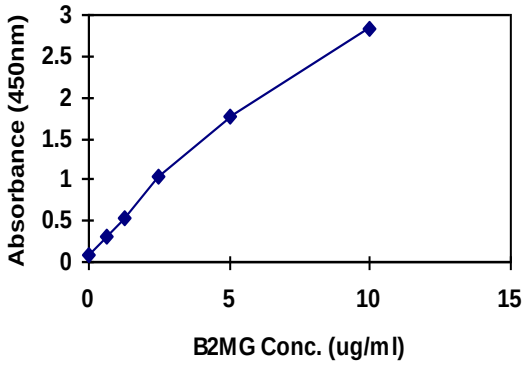
PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against β2-MG concentrations shown on 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 their own standard curve and patient data in each experiment.

β2-MG (µg/ml)	Absorbance (450 nm)
0	0.095
0.625	0.317
1.25	0.534
2.5	1.043
5.0	1.763
10.0	2.821



EXPECTED VALUES

It is recommended that each laboratory establish its own normal range. However, healthy individuals are expected to have β-2MG serum values between 0 - 2.0 µg/ml.[18]

The range of β-2 microglobulin concentration in serum was determined with the OriGene β-2 Microglobulin ELISA on a sample population of 66 healthy adults. The following was observed:
Mean = 1.42µg/ml
S.D. = 0.508 µg/ml
Mean ± 2SD = 0.4 – 2.8 µg/ml

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using 124 healthy and non-healthy patient samples, ranging in β2-MG concentration from 0.4 µg/ml to 17.2 µg/ml, demonstrated good correlation with a commercially available kit as shown below. Comparison between the OriGene β2-MG ELISA and the DPC Immulite® 2000 β2-MG kit provided the following data:

N = 124
Correlation coefficient = 0.9534
Slope = 0.9342 (variance = 0.0007, SE = 0.0268)
Intercept = 0.0498 (variance = 0.0095, SE = 0.0974)
OriGene Mean = 2.72 µg/ml
DPC Mean = 2.85 µg/ml

Similarly, an additional study using a second commercial kit was performed on 30 known patient samples with elevated levels of β2-MG (3.2 µg/ml to 65.8 µg/ml). The study demonstrated high correlation; the data is presented below.

N = 30
Correlation coefficient = 0.9932
Slope = 1.0097 (variance = 0.0005, SE = 0.0223)
Intercept = 0.4941 (variance = 0.1548, SE = 0.3934)
OriGene Mean = 12.86 µg/ml
Dade Behring = 12.25 µg/ml

2. Sensitivity

he minimum detectable concentration of the OriGene β2-MG ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be at least 0.1 µg/ml.

3. Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of three different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	20	20	20
Mean β2-MG (µg/ml)	0.92	1.63	4.43
Standard Deviation	0.100	0.108	0.228
Coefficient of Variation (%)	10.8%	6.6%	5.2%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of three different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3
Number of Replicates	24	24	24
Mean β2-MG (µg/ml)	0.83	2.03	4.90
Standard Deviation	0.065	0.097	0.350
Coefficient of Variation (%)	7.8%	4.8%	7.2%

4. Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known β2-MG levels were combined and assayed in duplicate. The mean recovery was 98.0%.

	Expected Concentration (µg/ml)	Observed Concentration (µg/ml)	% Recovery
	9.9	10.0	101%
	8.2	8.9	109%
	7.4	6.5	88%
	5.3	5.3	98%
	3.5	3.8	109%
	1.5	1.4	94%
	0.63	0.67	106%
			Mean: 100.7%

b. Linearity

Three patient samples were serially diluted to determine linearity. The mean recovery was 95.7%.

#	Dilution	Expected Conc. (µg/ml)	Observed Conc. (µg/ml)	% Expected
1.	Undiluted	-------	10.33	-----
	1:2	5.17	5.08	102%
	1:4	2.58	2.65	103%
	1:8	1.29	1.33	103%
	1:16	0.64	0.63	98%
				Mean = 102%
2.	Undiluted	----	6.95	----
	1:2	3.48	3.27	94%
	1:4	1.74	1.64	96%
	1:8	0.87	0.79	91%
	1:16	0.43	0.42	98%
				Mean = 95%
3.	Undiluted	----	7.16	----
	1:2	3.58	3.23	90%
	1:4	1.79	1.69	94%
	1:8	0.90	0.74	82%
	1:16	0.45	0.42	93%
				Mean = 90%

5. Specificity

The following hormones were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	PRODUCED COLOR INTENSITY EQUI. TO β2-MG IN SERUM (µgml)
Alpha-Fetoprotein (AFP)	1,000 ng/ml 5,000 ng/ml 10,000 ng/ml	0.0 0.0 0.0
Carcinoembryonic Antigen (CEA)	1,000 ng/ml 5,000 ng/ml 10,000 ng/ml	0.0 0.0 0.0
Ferritin	1,000 ng/ml 5,000 ng/ml 10,000 ng/ml	0.0 0.0 0.0
Prostate Specific Antigen (PSA)	1,000 ng/ml 5,000 ng/ml 10,000 ng/ml	0.0 0.0 0.0
Human IgG	5 g/l 10 g/l 25 g/l	0.0 0.0 0.0

6. Hook Effect

No high dose hook effect is observed in this assay at β2-MG levels up to 250 µg/ml.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. Controls containing sodium azide cannot be used. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. 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.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. Berggard, I and Bearn, AG, Isolation and properties of a low molecular weight B2-Globulin occurring in human biological fluids. *J Biol C*hem 243:4095-4103, 1968
2. Parker, KC and Strominger, JL, Sequence of human B2-microglobulin: A correction. *Mol Immunol*, 19:503-504, 1982.
3. Nakamuro, K, Tanigaki, N. and Pressman, D, Multiple common properties of human B2-microglobulin and the common portion fragment derived from HL-A antigen molecule. *Proc Natl Sci USA*, 70:2863-2865, 1973.
4. Grey, HM, Kubo, RT, Colon, SM, et.al., The small subunit of HL-A antigens in B2-microglobulin. *J Exp Med*, 138:1608-1612, 1973.
5. Peterson, PA, Rask, L and Lindblom, JB. Highly purified papain-solubilized HL-A antigens contain β2-microglobulin. *Proc Natl Acad Sci USA*, 71:35-39, 1974.
6. Evrin, P-E, and Wibell, L. The serum levels and urinary excretion of B2-microglobulin in apparently healthy subjects. *Scand J Clin Lab Invest*, 29:69-73, 1972.
7. Kjellstrom, T, and Piscator, M, Quantitative analysis of β2-microglobulin in urine as an indicator of renal tubular damage induced by cadmium. *Phadedoc Diagnostic Communications* No. 1, Pharmacia Diagnostics AB, Uppsala, Sweden, 1979.
8. Schentag, JJ, Suftin, TA, Plaut, ME, et. al., Early detection of aminoglycoside nephrotoxicity with urinary beta-2-microglobulin. *J Med*, 9:201-210, 1978.
9. Merle, LJ, Reidenberg, MN, Camacho, MT, et. al., Renal injury in patients with rheumatoid arthritis treated with gold. *Clin Pharmacol Ther*, 28:216-222, 1980.

10. Schardijn, G, Statius van Eps, LW, Swaek, AJG, et. al., Urinary B2-microglobulin in upper and lower urinary-tract infections. *Lancet*, 1:805-807, 1979.
11. Cooper, E., Forbes, M., Hammbling, M., Serum β2-microglobulin and C-reactive protein concentrations in viral infections. *J Clin Path*, 37:1140-3, 1984.
12. Manicourt, D., Brauman, H. and Orloff S., Plasma and urinary levels of β2-microglobulin in rheumatoid arthritis. *Ann Rheum Dis* 37:328-322, 1978.
13. Maury, CPJ, Helve, T and Sjoblom, C, Serum B2-microglobulin, sialic acid and C-reactive protein in systemic lupum erythematosus. *Rheumatol Int*, 2:145-149, 1982.
14. Mornex, JF, Revillard, JR, Vincent, C, et. al., Elevated serum B2-microglobulin levels and Clg-binding immune complexes in sarcoidosis. *Biomedicine* 31:210-213, 1979.
15. Beorchia, S, Vincent, C, Revillard, JP, et. al., Elevation of serum B2-microglobulin in liver diseases. *Clin Chem Acta*, 109:245-255, 1981.
16. Kaas Ibsen, K, Krabbe, S and Hesse, J, B2-microglobulin serum levels in infectious mononucleosis in childhood. *Acta Path Microbiol Scand*, Sec. C, 89:205-208, 1981.
17. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", (1984).
18. "Clinical Guide to Laboratory Tests". Edited by N.W. Tietz, 3rd Edition. W.B. Saunders Company, Philadelphia, PA. 19106 (1995).

TECHNICAL CONSULTATION

OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200
Rockville, MD 20850

Phone: 1.888.267.4436
Fax: 301-340-9254
Email: techsupport@origene.com
Web: www.origene.com

091815

For Research Use Only
Not for use in diagnostic procedures