



β 2-MG ELISA

Catalog Number EA-0108

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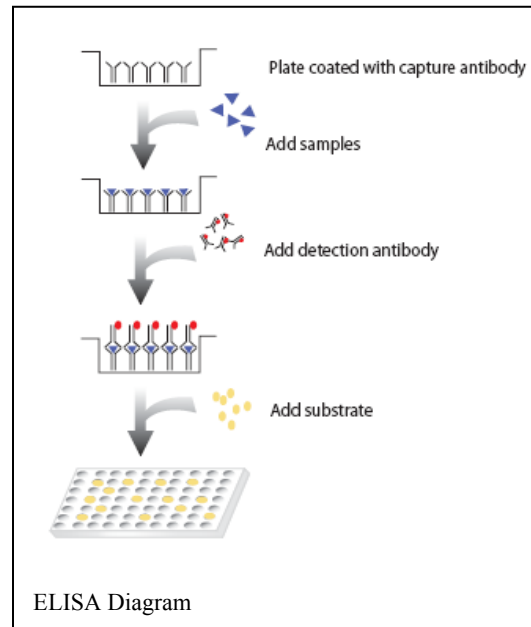
Introduction

Human β 2-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). It is mainly found on the surface of white blood cells. Increased production or destruction of these cells causes Beta₂-microglobulin levels in the blood to increase. Elevated β 2-MG level in blood is associated with certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and multiple myeloma or kidney disease. Beta₂-microglobulin normally is filtered out of the blood by the kidney's glomeruli, only to be partially reabsorbed back into the blood when it reaches the kidney's tubules. In glomerular kidney disease, the glomeruli can't filter it out of the blood, so levels increase in the blood and decrease in the urine. In tubular kidney disease, the tubules can't reabsorb it back into the blood, so urine levels rise and blood levels fall (6).

The Signosis β 2-MG ELISA provides a sensitive and reliable assay for the measurement of β 2-microglobulin in human serum. The kit features a standard range of 0.625 to 10 μ g/ml and will determine a minimum detectable concentration.

Principle of the assay

The β 2-MG is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a monoclonal anti- β 2-MG antibody for solid phase immobilization on the microtiter wells. A sheep anti-anti- β 2-MG antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with these antibodies, resulting in β 2-MG being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of β 2-MG is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kits:

- 96 well microplate coated with murine monoclonal anti- β 2-MG
- sheep anti- β 2-MG conjugated to horseradish peroxidase (HRP).
- Reference Standard Set containing 0, 0.625, 1.25, 2.5, 5 and 10 μ g/ml β 2-MG in sample diluent, pre-diluted 101-fold lyophilized.
- Sample Diluent
- TMB Reagent
- Stop Solution

Materials required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Specimen Collection and Handling

Serum should be separated from the red blood cells as soon as possible. Specimens should be stored for up to 48 hours or -20°C for up to 6 months prior to assay.

Preparation for Assay

- All reagents should be taken to room temperature (18-25 °C) before use.
- 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 will be stable for up to 30 days when stored sealed at 2-8 °C.

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 10µl serum with 1.0 ml Sample Diluent (101 fold dilution). Do not dilute the standards, they have already been pre-diluted 101 fold.

1. Add 5µl of standards, diluted specimens, and diluted controls into appropriate wells.
2. Add 200 µl of Sample Diluent into each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
3. Incubate at 37°C for 30 minutes.
4. Remove the incubation mixture by flicking plate contents into a waste container.
5. Rinse and flick the microtiter wells 5 times with deionized or distilled water.
6. Tap the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 200 µl of Enzyme Conjugate Reagent into each well.
8. 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 100µl 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 100µl of Stop Solution 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.

References:

1. Berggard, I and Bearn, AG, Isolation and properties of a low molecular weight B2-Globulin occurring in human biological fluids. *J Biol Chem* 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.

Example of standard curve

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

