Human Beta-2 Microglobulin (B2M)
ELISA Kit Cat. #. 0020

For Quantitative Determination of Beta-2 Microglobulin In Human Serum
For In Vitro Research Use Only
Kit Contents: (reagents for 96 tests)

Components	96 tests
Monoclonal Anti-human B2M coated microwell strip plate (96 wells), Cat. # 0021	1 plate
Human B2M **Std. A**, 0 ng/mL, 50 ml Cat. # 0022	1 bottle
Human B2M **Std. B**, 2.5 ng/mL, 0.5 ml Cat. # 0023	1 Vial
Human B2M **Std. C**, 5 ng/mL, 0.5 ml Cat. # 0024	1 Vial
Human B2M **Std. D**, 20 ng/mL, 0.5 ml Cat. # 0025	1 Vial
Human B2M **Std. E**, 50 ng/mL, 0.5 ml Cat. # 0026	1 Vial
Human B2M **Std. F**, 150 ng/mL, 0.5 ml Cat. # 0027	1 Vial
Anti-B2M-HRP Conjugate, 11 ml Cat. # 0028	1 bottle
Wash Buffer (100X); 10 ml, **dilute 1:100 with distilled water**; Cat# W-100	1 bottle
HRP substrate Solution, Cat. # TMB-20	1 bottle
Stop solution, 10 ml, Cat. # T-10	1 bottle
Instruction Manual, M - 0 0 2 0	1

Introduction

Human Beta-2 Microglobulin (B2M) is a small (11.0 kDa) polypeptide that forms the invariant subunit of the class I HLA-antigens (Human Lymphocyte Antigen) on the cell membrane (1). Increased concentrations of B2M in serum have been found in patients with renal diseases, malignancies (especially of lymphatic origin) and infectious diseases, e.g., AIDS. B2M is the most powerful single prognostic variable in multiple myeloma, it is also a good predictor for acute rejection in renal transplantation, and furthermore the measurement of B2M is very useful for detecting and monitoring the changes in the glomerular filtration rate in the disease of diabetic nephropathy. Various immunoassays, including radioimmuno-assay (RIA), turbidimetry, Immunoflurometric assay, nephelometry and radial

immunodiffusion were developed too measure B2M is serum. ADI's B2M ELISA kit provides a sensitive assay for B2M in serum.

PRINCIPLE OF THE TEST

Human Beta-2 microglobulin (B2M) ELISA kit is based on simultaneous binding of human B2M from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of B2M present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of B2M in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 ul) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic Intl., Inc. B2M ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions. Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20oC for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

EXPECTED VALUES

It is recommended that each laboratory determine its own normal and abnormal range. A clinical study of test was conducted for 100 normal serum showed a range of 0.8-2.5 ug/ml (99% samples).

HIGH DOSE HOOK EFFECT

This kit is designed to avoid hook effect. However, samples with >200 ng/ml should be further diluted to get accurate values.

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SPECIFICITY

The specificity of B2M ELISA kit was determined by measuring interference from high concentrations of hemoglobin (20 g/dl), bilirubin (10 mg/dl); Triglyceride (220 mg/dl), albumin (5 g/dl), uric acid (5mg/dl), and calcium (10 mg/dl).

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8oC until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two month at 2-8oC. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Dispense 200-300 ul of wash buffer to all wells. Mix for 5 seconds and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

1. Label or mark the microtiter well strips to be used on the plate. Do not dilute standards. Dilute sample 1:100 (5 ul in 495 ul of zero std or sample diluent).

2. Pipet **10 ul of standards**, and diluted control, and serum samples into appropriate wells in *duplicate*. Add **100 ul antibody-enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **60 minutes** at room temperature.

3. Wash the plate 5X with 1X wash buffer (300 ul/wash). We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. **Dispense 100 ul TMB substrate per well** . Mix gently, cover the plate and **incubate for 15 min at room temp**. Blue color develops into standard and all positive wells.

5. Stop the reaction by adding **50 ul of stop solution** to all wells at the same timed intervals as in step 6. Mix gently. Blue color turns yellow. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES- Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4oC. Addition of the HRP substrate

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solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards, control, and samples. Draw the standard curve on log-log graph paper by plotting net absorbance values of standards against appropriate protein concentrations. Read off the B2M concentrations of the control and patient samples. Sample values must be multiplied by the dilution factor. It is possible to dilute sample more (>1:100 for samples that are >200 ng/ml) or less (1:50 for samples that are in the lower range 0-5 ng/ml to enhance sensitivity).

Testing of other Biological Fluids Species Crossreactivity

This kit is primarily designed to test human serum samples. It is possible to use the plasma and other biological fluids including cell culture medium. However, the sample dilutions must be adjusted according to the expected concentrations or unknown samples be tested at several dilutions to determine the optimum range. Crossreactivity with B2M from other species was determined by testing sera from various species.

Bovine, Cat, Dog, Donkey, Hamster, Horse, Mouse, Rat B2M

No significant crossreactivity was detected using 1:5 diluted sera.

Monkey B2M

At 1:5 dilution monkey sera yielded values equal to or higher than the equivalent human serum. It is concluded that the human kit has equal reactivity with the monkey beta-2 microglobulin. Due to the lack of purified B2M or known standards, it is not possible to determine absolute crossreactivity.

General References:

Viedma JA et al (1992) Clin. Chem. 12, 2464;
Bernard AM (1982) CLin. Chim. Acta 126, 1;
Tienhara A (1990) Clin. Chem. 36, 1961;
Hemmingsen L (1985) Clin. Lab Invest. 45, 367;
Bjerrum OW (1986) Clin. Chim. Acta (1986) 155, 69

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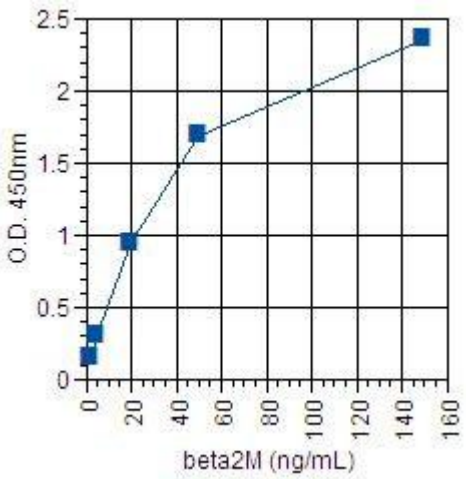
WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated Conc. (ng/ml)
A1, A2	**Std. A** (0 ng/ml)	0.002	
B1, B2	**Std. B** (2.5 ng/ml)	0.145	
C1, C2	**Std. C** (5.0 ng/ml)	0.325	
D1, D2	**Std. D** (20 ng/ml)	0.945	
E1, E2	**Std. E** (50 ng/ml)	1.745	
F1, F2	**Std. F** (150 ng/ml)	2.40	
G1, G2	**Sample 1**	0.860	18

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical std. assay curve (do not use this for calculating sample values)

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT - Based on sixteen replicates determinations of the zero standard, the minimum concentration of human B2M detected using this assay is ~ 0.5 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

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Intra-assay precision:

	Pool A	Pool B	Pool C
N	12	12	12
Mean (ng/ml)	24	32	180
S.D (ng/ml)	1.9	1.5	1.6
C.V (%)	7.9	4.6	8.9

Inter-assay precision:

	Pool A	Pool B	Pool C
N	12	12	12
Mean (ng/ml)	26	37	178
S.D (ng/ml)	2.7	3.4	12
C.V (%)	10.3	9.1	6.7

LINEARITY

Samples with known concn were spiked with known conc (diluted 1:2 v/v) and B2M values determined.

Initial (ng/ml)	Spiked (ng/ml)	Expected (ng/ml)	Observed (ng/ml)	Recovery (%)
10	20	15	16	106
10	100	55	55	100
10	200	106	115	99
44	20	32	32	100
44	100	72	75	104
44	200	122	120	99

A serum sample with different concentration of B2M was diluted with series of B2M-free serum. The dilutions were tested for B2M.

Initial (ng/ml)	Dilution (ng/ml)	Expected (ng/ml)	Observed (ng/ml)	Recovery (%)
52	1:2	26	24	92
	1:4	13	13	100
	1:8	6.5	6.2	95
	1.:16	3.2	4	12
98	1:2	49	44	90
	1:4	24	22	93
	1:8	12	13	108
200	1:2	100	98	98
	1:4	50	55	110
	1:8	25	26	104

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