

ELISA kits available from ADI (see details at the web site)

#0010	Human Leptin		
#200-120-AGH	Human globular Adiponectin (gAcrp30)		
#0700	Human Sex Hormone Binding Glob (SHBG)		
#0900	Human IGF-Binding Protein 1 (IGFBP1)		
#1000	Human C-Reactive Protein (CRP)		
#100-110-RSH	Human Resistin /FIZZ3		
#100-140-ADH	Human Adiponectin (Acrp30)		
#100-160-ANH	Human Angiogenin		
#100-180-APH	Human Angiopoietin-2 (Ang-2)		
#100-190-B7H	Human Bone Morphogenic Protein 7 (BMP-7)		
#1190	Human Serum Albumin	#1200	Human Albumin (Urinary)
#1750	Human IgG (total)	#1760	Human IgM
#1800	Human IgE	#1810	Human Ferritin
#1210	Human Transferrin (Tf)	#0020	Beta-2 microglobulin
#1600	Human Growth Hormone (GH)		

#0060	Human Pancreatic Colorectal cancer (CA-242)		
#1820	Human Ovarian Cancer (CA125)	#1830	Human CA153
#1840	Human Pancreatic & GI Cancer (CA199)		
#1310	Human Pancreatic Lipase		
#1400	Human Prostatic Acid Phosphatase (PAP)		
#1500	Human Prostate Specific Antigen (PSA)	#1510	free PSA (fPSA)
#0500	Human Alpha Fetoprotein (AFP)		
#0050	Human Neuron Specific Enolase (NSE)		

#0030	Human Insulin	#0040	Human C-peptide
#0100	Human Luteinizing Hormone (LH)		
#0200	Human Follicle Stimulating Hormone (FSH)		
#0300	Human Prolactin (PRL)		
#0400	Human Chorionic Gonadotropin (HCG)	#0410	HCG-free beta

#0600	Human Thyroid Stimulating Hormone (TSH)		
#1100	Human Total Thyroxine (T4)	#1110	Human Free T4 (fT4)
#1650	Human free triiodothyronine (fT3)	#1700	Human T3 (total)

#1850	Human Cortisol	#1860	Human Progesterone
#1865	Human Pregnenolone	#1875	Human Aldosterone
#1880	Human Testosterone	#1885	Human free Testosterone
#1910	Human Androstenedione	#1920	Human Estradiol
#1925	Human Estrone	#1940	Dihydrotestosterone (DHT)
#1950	Human DHEA-sulphate (DHEA-S)		
#3400	Human serum Neopterin		

#3000	Human Rheumatoid Factors IgM (RF)		
#3100	Human anti-dsDNA		
#3200	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-0020

Human Beta-2 Microglobulin (B2M)

ELISA Kit Cat. # 0020, 96 Tests

For Quantitative Determination of
Beta-2 Microglobulin in Human Serum



For In Vitro Research Use Only



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Human Beta-2 Microglobulin (B2M) ELISA KIT

Cat. No. 0020; 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 ug/ml , 1.5 ml Cat. # 0022A	1 vial
Human B2M Std. B, 0.75 ug/ml , 1.5 ml Cat. # 0022B	1 Vial
Human B2M Std. C, 1.5 ug/ml , 1.5 ml Cat. # 0022C	1 Vial
Human B2M Std. D, 3 ug/ml , 1.5 ml Cat. # 0022D	1 Vial
Human B2M Std. E, 6 ug/ml , 1.5 ml Cat # 0022E	1 Vial
Human B2M Std. F, 12 ug/ml , 1.5 ml, Cat # 0022F	1 Vial
Low (#0022-CL) & High controls (#0022-CH) exact values printed on vials, 1.5 ml/vial	2 Vials
Anti-B2M-HRP Conjugate, 15 ml, # 0023	1 bottle
Sample Buffer(5X) 20 ml, #0024 dilute with distilled water in 80 ml	1 bottle
Wash Buffer (50X); 20 ml, dilute with distilled water to final vol 1000 ml; #W-10	1 bottle
HRP substrate Solution, 15 ml, # TMB-20	1 bottle
Stop solution, 15 ml, Cat. # ST-10	1 bottle
Instruction Manual, M - 0 0 2 0	1

Intended Use

ADI's B2M ELISA kit provides a sensitive assay for B2M in serum, plasma, or urine. For in vitro research use only (RUO).

Introduction

Human Beta-2 Microglobulin (B2M) is a small (~12 kDa) polypeptide that forms the invariant subunit of the class I HLA-antigens (Human Lymphocyte Antigen) on the cell membrane. 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, Immunofluorometric assay, nephelometry and radial immunodiffusion were developed to measure B2M in serum.

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT - Based on sixteen replicates determinations of the zero standard, the minimum concentration of human B2M detected using this assay is ~ 0.1 ug/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Intra-assay precision:

	Pool A	Pool B	Pool C
Mean (ug/ml)	1.6	7.2	12.5
C.V (%)	4.2	2.6	3.6

Inter-assay precision:

	Pool A	Pool B	Pool C
Mean (ug/ml)	1.7	7.5	13.1
C.V (%)	4.9	3.8	4.9

LINEARITY

Serum sample with different concentration of B2M was serially diluted with the assay buffer or B2M-free serum. The dilutions were tested for B2M and the recover was 95-115%.

EXPECTED VALUES

It is recommended that each laboratory determine its own normal and abnormal range. A clinical study of test was conducted for normal serum and urine.

Urine samples	0-0.3 ug/ml
Serum or plasma	0-0.30 ug/ml

Detection Limit:

Functional sensitivity was determined to be: 0.1 µg/ml

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.

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). Antibodies used in the kit are specific for beta-2M and do not recognize other serum or urine proteins.

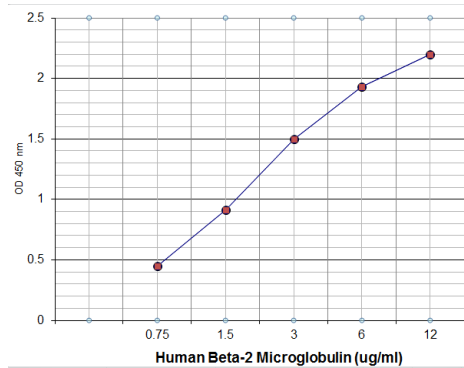
Species crossreactivity

No significant crossreactivity was detected using 1:5 diluted sera from Bovine, Cat, Dog, Donkey, Hamster, Horse. 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. It has been reported that ADI human B2M ELISA kit also detected mouse and rat beta-2 microglobulin (see published papers on page 4).

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated Conc. (ug/ml)
A1, A2	Std. A (0 ug/ml)	0.025	
B1, B2	Std. B (0.75 ug/ml)	0.532	
C1, C2	Std. C (1.5 ug/ml)	0.910	
D1, D2	Std. D (3.0 ug/ml)	1.506	
E1, E2	Std. E (6.0 ug/ml)	1.934	
F1, F2	Std. F (12.0 ug/ml)	2.207	
G1, G2	Sample 1	0.95	1.52

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values.



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

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the standard A (zero B2M) from the mean absorbance values of standards, control, and samples. Draw the standard curve on lin-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 >12 ug/ml) or less (for samples that are in the lower range to enhance sensitivity). If using ELISA software then we recommend 4-parameter fit with lin-log coordinates.

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 Proclin-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 -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. Do not use heat inactivated samples. Collect either morning urine. Avoid repeated freeze and thaw of samples. Samples may have to be diluted before the assay (see sample preparation).

Reagent and Sample Preparation

Sample Buffer: Dilute stock (5x) with water (20 ml stock in 80 ml water). Store at 2-8C until the expiration date. The diluted sample buffer will be used to dilute the samples.

Wash buffer (50X). Dilute 1:50 with water (50 ml stock in 950 water). Store at 2-8C until the expiration date.

Sample preparation. Dilute all urine samples 1:10 with the sample buffer (30 ul sample in 290 ul buffer). Dilute serum or plasma 1:100 (10 ul sample in 990 ul sample buffer). All samples must be diluted prior to the assay in a clean dilution plate to allow rapid sample transfer to the coated plate. Do not dilute standards or controls.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C 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 2-8°C. 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 (1:50) with distilled water. Prepare 1X solution by diluting **1:50** (20 ml concentrate in 980 ml water). Store diluted stock at 4°C. **Dilute Sample buffer 1:5** with distilled water (20 ml stock in 80 ml water). Dilute all samples 1:10 (adjusted for sample concentration prior to start of the assay with the sample diluent).

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate. Do not dilute standards. **Dilute samples accordingly (see sample preparation).**
2. Pipet **100 ul of ready-to-use-standards and controls**, and diluted samples into appropriate wells in *duplicate*. Cover the plate and **incubate for 30 minutes** at room temperature (25-28°C).
3. **Wash the plate 3X 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. Add **100 ul antibody-enzyme conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and **incubate for 15 minutes** at room temperature.
5. **Wash the plate 3X with 1X wash buffer** (300 ul/wash) as in step 3 above.
6. **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.
7. Stop the reaction by adding **100 ul of stop solution** to all wells at the same timed intervals. Mix gently. Incubate for 5 minutes at room temp. 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 4°C. Addition of the HRP substrate 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.

Testing of other Biological Fluids Species Crossreactivity

This kit is primarily designed to test human serum samples, plasma or urine. It is possible to use 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.

Quality Control

Standards and controls must perform as expected.

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

(2) Citations of ADI's B2M ELISA kit #0020 (see web site for updated list)

Wani MA	2006	PNAS 103, 5084-5089
Schnapp LM	2006	Am. J. Pathol., Jul 2006; 169: 86 - 95.
Martin PM	2006	Invest. Ophthalmol. Vis. Sci., Oct; 47: 4238 – 4244
Wang M	2007	Blood, Jun 2007; 109: 5455 - 5462
Rabbani N	2007	Kidney International 72, 1113-1121
Auf G	2010	PNAS, 10.1073/pnas.0914072107.
Matsubara J	2011	Cancer Epidemiol. Biomarkers Prev., 20: 160 - 171.