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 Pregnolone	**#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-0360-0B6

Vitamin B6 ELISA KIT

Cat. # 0360-0B6, 96 Tests

For Quantitative of vitamin B6 (VB6) in Serum, Plasma, and Biological Fluids (culture supernatant).

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 West • San Antonio• Texas 78249 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: Techsupport@4adi.com

Web Site: www.4adi.com

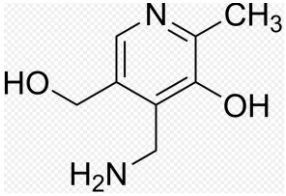
Vitamin B6 ELISA Kit Cat. # 0360-0B6

Kit Components	96 tests
B6-**coated strip plate** (96 wells), #0360B6-1	1 plate
Reference **Standard**, # 0360B6-2 (**100 ng/ml** stock to be used for preparing 100, 50, 25, 12.5, 6.25, 3.13, and 1.56, 0 ng/ml additional standards).	2 Vials
Sample Diluent, 20 ml, #0360B6-3	1 Bottle
Biotinylated Detection ab (100X), 120 µl, #0360B6-4	1 Vial
Biotinylated Detection ab Diluent, 14 ml, #0360B6-5	1 Bottle
HRP-conjugate (100X), 120 µl, # 0360B6-6-	1 Vial
HRP-Conjugate Diluent, 14 ml, #0360B6-7	1 Bottle
Wash buffer concn. (25X) , #0360-WB, 30 ml	1 Bottle
TMB substrate, # 0360-TMB, 10 ml	1 Bottle
Stop solution, #0360-ST	1 Bottle
Plate sealer	2 pieces
Instruction Manual, M-0360-0B6	1

Intended Use

ADI's Vitamin B6 ELISA kit is used for the quantitative detection of vitamin B6 in serum, plasma and other biological fluids (culture supernatant). **For in vitro research use only (RUO).**

Introduction



Vitamin B6 refers to a group of chemically very similar compounds which can be interconverted in biological systems. Vitamin B6 is part of the vitamin B complex group, and its active form, Pyridoxal 5'-phosphate (PLP) serves as a cofactor in many enzyme reactions in amino acid, glucose, and lipid metabolism. **Vitamin B6** is also called **pyridoxine**. It is involved in the process of making serotonin and norepinephrine, which are chemicals that transmit signals in the brain. Vitamin B6 is also involved in the formation of myelin, a protein layer that forms around nerve cells.

Vitamin B6 deficiency in adults may cause health problems affecting the nerves, skin, mucous membranes, and circulatory system. In children, the central nervous system is also affected. Deficiency can occur in people with kidney failure complications, alcoholism, liver scarring, overactive thyroid, problems with absorbing nutrients, and heart failure, as well as those taking certain medications. Mild deficiency of vitamin B6 is common.

Major sources of vitamin B6 include cereal grains, legumes, vegetables (carrots, spinach, peas, and potatoes), milk, cheese, eggs, fish, liver, meat, and flour. Vitamin B6 is often used with other B vitamins in vitamin B complex formulas.
High blood levels of the amino acid homocysteine may be a risk factor for heart disease. Taking vitamin B6 supplements with other B vitamins (folic acid and vitamin B12)

Important Note:

1. **ELISA Plate**: The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.

2. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.

3. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.

4. **Washing**: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.

5. **Reagent Preparation**: As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10µL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into a small pack according to the amount of each assay, keep them at -20～-80℃ and avoid repeated freezing and thawing.

6. **Reaction Time Control:** Please control reaction time strictly following this product description!

7. **Substrate:** Substrate Solution is easily contaminated. Please protect it from light.

8. **Stop Solution:** As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.

9. **Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.

10. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.

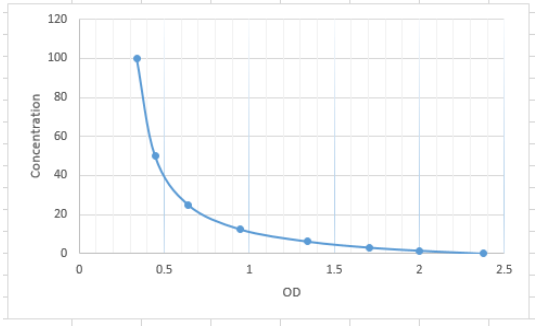
11. Do not use components from different batches of kit (washing buffer and stop solution can be an exception).

12. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450nm	Calculated concn.
A1, A2	Std. A (100 ng)	0.338	
B1, B2	Std. B (50 ng)	0.447	
C1, C2	Std. C (25 ng)	0.641	
D1, D2	Std. D (12.5 ng)	0.945	
E1, E2	Std. E (6.25 ng)	1.33	
F1, F2	Std. F (3.13 ng)	1.708	
G1, G2	Std. G (1.56 ng)	1.999	
H1, H2	Std. H (0 ng)	2.379	

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.



7_ADI-ELISA-graph

Calculation of results:

Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Sensitivity

The minimum detectable dose of VB6 is **0.94 ng/mL** (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest protein concentration that could be differentiated from zero).

Detection Range: 1.56- 100 ng/mL

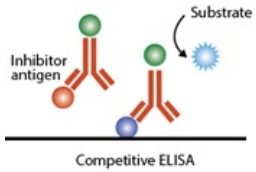
Specificity This kit recognizes natural and recombinant VB6. No significant cross-reactivity or interference between VB6 and analogues was observed.

Note : Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between VB6 and all the analogues.

Repeatability Coefficient of variation were<10%.

has been shown to be effective for lowering homocysteine levels. Vitamin B6 has been studied for the treatment of many conditions, including anemia (low amounts of healthy red blood cells), vitamin B6 deficiency, certain seizures in newborns, and side effects of the drug cycloserine.

PRINCIPLE OF THE TEST



Vitamin B6 ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with VB6. During the reaction, VB6 in the sample or standard competes with a fixed amount of VB6 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to VB6. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of VB6 in the samples is then determined by comparing the OD of the samples to the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (50-200 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

SPECIMEN COLLECTION AND STORAGE

Samples should be clear and transparent and be centrifuged to remove suspended solids. **Serum**: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for ELISA assay!

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Tissue homogenates: You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8℃. Collect the supernatant and carry out the assay immediately.

Notes:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Sample preparation:

1. The user should calculate the possible amount of the samples needed in the whole test. Reserving sufficient samples in advance is recommended.
2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the kit is necessary.
3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected Elisa results due to the impacts of certain chemicals.
4. Due to the possibility of mismatching between antigen from other origins and antibodies used in our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.
5. Influenced by factors including cell viability, cell number or sampling time, molecular from cells culture supernatant may not be detected by the kit.
6. Grossly hemolyzed samples are not suitable for use in the assay.
7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENTS PREPARATION FOR THE ASSAY:

1X Biotinylated Detection Ab – Calculate the required amount before experiment (50 µL/well). Prepare 500 ul/strip or 5 ml for full plates. Centrifuge the stock tube before use, Dilute the 100x Biotinylated Detection Ab 1:100 (10 ul stock in 100 ul of diluent or 100 ul stock in 10 ml diluent) with Biotinylated Detection Ab Diluent. Do not store 1x antibody solution beyond the assay date.

1X HRP Conjugate – Calculate the required amount before experiment (100µL/well). Prepare 1 ml/strip or 10 ml for full plates. Dilute the 100X HRP Conjugate 1:100 to the working concentration using HRP Conjugate Diluent (10 ul stock in 100 ul of diluent or 100 ul stock in 10 ml diluent). Do not store 1x conjugate solution beyond the assay date.

Wash Buffer (25X): Dilute the wash buffer with distilled water (dissolve content of 1 bottle (30 ml) into 750 ml water. Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8oC.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw.

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. The unused strips should be stored in a sealed bag at 4^{O}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 wells the same by adding the reagents in identical sequence.

Standard preparation:

Prepare standard within 15 minutes before use. Centrifuge the stock tube in a microfuge for 1 minute, and reconstitute the Standard with 1.0 mL of Reference Standard Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of **100 ng/mL**. Then make 2-fold serial dilutions as needed (making serial dilution in the wells directly is not permitted). The recommended concentrations are as follows: 100, 50, 25, 12.5, 6.25, 3.13, and 1.56, 0 ng/mL. The Reference Standard & Sample Diluent serves as the zero (0 ng/mL). Need 50 ul standards/well or 100 ul in duplicate (prepare 150 ul of each standard for the test).

	Volume	Std. Diluent	Final conc.	Std Name
Stock std. 100 ng/ml	150 ul	150 ul	50 ng/ml	F
Std F	150 ul	150 ul	25 ng/ml	E
Std E	150 ul	150 ul	12.5 ng/ml	D
Std D	150 ul	150 ul	6.25 ng/ml	C
Std C	150 ul	150 ul	3.13 ng/ml	B
Std B	150 ul	150 ul	1.56 ng/ml	A
Blank	-	150 ul	0	blank

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE). Prepare working solutions of biotin-antibody, HRP conjugate and wash buffer (see page 3). Bring all reagents and solutions to room temp. (25-28oC).*

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipette **50 µl** of stds., control, and samples in duplicate into appropriate wells. Pipette **50 µl of 1X Biotinylated detection ab working solution** into each well. Mix gently for 5-10 secs.
3. Cover the plate and incubate for **45 min at 37 °C**.
4. Aspirate and wash the wells 3 times with 300 **µl** of diluted wash buffer. 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
5. Add **100 µl of 1X HRP** conjugate into each well. Mix gently and incubate for **30-min** at **37 °C**.
6. Aspirate and wash the wells 5 times with 300 **µl** of diluted wash buffer as in step 4.
7. Add **90 µl of (TMB**) substrate into **each well.** Mix gently. Cover the plate and incubate for **15 minutes** at **37 °C**.
8. Stop the reaction by adding **50 µl** of stopping solution to **all wells** at the same timed intervals. Mix gently.
9. Measure the absorbance at 450 nm using an ELISA reader within 15 minutes.