Version 17-12/25

User's Manual

Human Vitamin B6 ELISA kit

REF **DEIA-BJ724**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Human Vitamin B6 ELISA kit is a solid-phase ELISA designed for the quantitative determination of the Vitamin B6. This ELISA kit is for research use only, not for therapeutic or diagnostic applications.

General Description

This assay is based on the competitive EIA principle. Each well of the supplied microtiter plate has been pre-coated with antigen. Standards or samples are added to the wells as well as a Biotin-conjugated detection antibody. The free antigens (in the standards or samples) and antigens bound to the plate compete to bind to the detection antibody. All but the bound Biotin-conjugated detection antibody is washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added which binds to the biotin. Unbound HRP-conjugate is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration. In contrast to typical Sandwich ELISA assays, in competition assay the greater the amount of antigen in the sample, the lower the color development and optical density reading.

Reagents And Materials Provided

1. Coated 96-well Strip Plate 1
2. Standard (Lyophilized) 2 vials
3. Reference Standard & Sample Diluent 1 vial × 20 ml
4. Biotinylated Detection Antibody (100x) 1 vial × 120 µl
5. Detection Antibody Diluent 1 vial × 14 ml
6. HRP Conjugate (100x) 1 vial × 120 µl
7. HRP Conjugate Diluent 1 vial × 14 ml
8. Wash Buffer (25x) 1 vial × 30 ml
9. TMB Substrate 1 vial × 10 ml
10. Stop Solution 1 vial × 10 ml
11. Adhesive Plate Sealers 4
12. Instruction Manual 1

Materials Required But Not Supplied

1. Microplate reader with 450nm wavelength filter
2. High-precision pipette and sterile pipette tips
3. Eppendorf tubes

4. 37°C incubator
5. Deionized or distilled water
6. Absorbent paper

Storage

Store all kit components at 4°C. Store Coated 96-well Strip Plate, Standard, Biotinylated Detection Antibody and HRP Conjugate at -20°C if the kit will not be used within 1 month. The Substrate should never be frozen. Once individual reagents are opened it is recommended that the kit be used within 1 month. Unused Strip Plate wells should be stored at -20°C in a sealed bag containing desiccant in order to minimize exposure to moisture. Do not use the kit beyond its expiration date.

Specimen Collection And Preparation

This assay is intended for use with samples such as Cell Culture Supernatants, Cell Lysates, Plasma, Serum, and Tissue Homogenates. The sample collection protocols below have been provided for your reference.

Breast Milk - Centrifuge samples for 20 minutes at 1000×g to remove particulates. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Cell Lysates - Collect and pellet the cells by centrifugation and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication 4 times. Alternatively freeze the cells to -20°C and thaw to room temperature 3 times. Centrifuge at 1500×g for 10 minutes at 2 - 8°C to remove cellular debris. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Erythrocyte Lysates - Centrifuge whole blood for 20 minutes at 1000×g to pellet the cells and remove the supernatant. Wash the cells 3 times with PBS* then resuspend in PBS*. Freeze (-20°C)/thaw (room temperature) the cells 3 times. Centrifuge at 5,000×g for 10 minutes at 2-8°C to remove cellular debris. Collect the supernatant for assaying. Erythrocyte lysates must be diluted with Reference Standard & Sample Diluent before running. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Platelet-Poor Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2–8°C within 30 minutes of collection. It is recommended that samples should be centrifuged for 10 minutes at 10,000×g for complete platelet removal. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Sperm and Seminal Plasma – Allow semen to liquefy at room temperature or 37°C. After liquefaction, centrifuge at 2,000×g for 10-15 minutes. Collect seminal plasma supernatant for assaying. Wash the precipitated protein 3 times with PBS* then resuspend in PBS*. Lyse the cells by ultrasonication then centrifuge at 2,000×g for 10-15 minutes. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Tissue Homogenates–Because preparation methods for tissue homogenates vary depending upon tissue type, users should research tissue specific conditions independently. The following is one example only. Rinse tissues in PBS* to remove excess blood and weigh before homogenization. Finely mince tissues and homogenize them in 5-10 mL of PBS* with a glass homogenizer on ice. Lyse the cells by ultrasonication or freeze (-20°C)/thaw (room temperature) 3 times. Centrifuge homogenate at 5000 ×g for 5 minutes. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter and collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Cell culture supernatants, cerebrospinal, follicular, and lung lavage fluids, saliva, sweat, tears, and other biological fluids - Centrifuge samples for 20 minutes at 1000 ×g to remove particulates. Collect the supernatant for assaying. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

* 1xPBS (0.02 mol/L pH7.0-7.2)

Sample Collection Notes

1. Recommend that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. CD is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

Plate Preparation

The following is an example of how to layout a study. A dilution series of the positive control Standard should be run in duplicate or triplicate, with the last well in each series being the negative control blank. Samples should also be run in duplicate or triplicate. Unknown samples should be run as a dilution series in order to identify the optimal dilution that produces an OD reading within the OD range of the positive control Standard dilution series.

Example 1: Standard Curve and dilution series of an unknown sample.

	1	2	3	4
A	Standard Dilution 1	Standard Dilution 1	Sample (1:1)	Sample (1:1)
B	Standard Dilution 2	Standard Dilution 2	Sample (1:10)	Sample (1:10)
C	Standard Dilution 3	Standard Dilution 3	Sample (1:100)	Sample (1:100)
D	Standard Dilution 4	Standard Dilution 4	Sample (1:1k)	Sample (1:1k)
E	Standard Dilution 5	Standard Dilution 5	Sample (1:10k)	Sample (1:10k)
F	Standard Dilution 6	Standard Dilution 6	Sample (1:100k)	Sample (1:100k)
G	Standard Dilution 7	Standard Dilution 7	Sample (1:1,000k)	Sample (1:1,000k)
H	Negative Control	Negative Control	Sample (1:10,000k)	Sample (1:10,000k)

Example 2: Standard Curve and samples run in duplicate.

	1	2	3	4
A	Standard Dilution 1	Standard Dilution 1	Sample A	Sample E
B	Standard Dilution 2	Standard Dilution 2	Sample A	Sample E
C	Standard Dilution 3	Standard Dilution 3	Sample B	Sample F
D	Standard Dilution 4	Standard Dilution 4	Sample B	Sample F
E	Standard Dilution 5	Standard Dilution 5	Sample C	Sample G
F	Standard Dilution 6	Standard Dilution 6	Sample C	Sample G
G	Standard Dilution 7	Standard Dilution 7	Sample D	Sample H
H	Negative Control	Negative Control	Sample D	Sample H

Reagent Preparation

Bring all reagents to room temperature (18-25°C) before use.

Standard Preparation

The following are instructions for the preparation of a Standard dilution series which will be used to generate the standard curve. The standard curve is then used to determine the concentration of target antigen in unknown samples (see the Calculation of Results section). The following will prepare sufficient volumes to run the Standard dilution series in duplicate. Reconstituted Standard and prepared standard dilutions should be used immediately and not stored for future use.

Standard Stock Solution (100 ng/ml): Briefly centrifuge the vial to ensure that all lyophilisate is collected at the bottom of the vial. Reconstitute 1 tube of lyophilized Standard with 1.0 ml of Reference Standard & Sample Diluent. Incubate at room temperature for 10 minutes with gentle agitation (avoid foaming).

D1 (100 ng/ml):

Pipette 500 µl of Stock Standard into 0 µl of Reference Standard & Sample Diluent

D2 (50 ng/ml):

Pipette 250 µl of D1 into 250 µl of Reference Standard & Sample Diluent

D3 (25 ng/ml):

Pipette 250 µl of D2 into 250 µl of Reference Standard & Sample Diluent

D4 (12.5 ng/ml):

Pipette 250 µl of D3 into 250 µl of Reference Standard & Sample Diluent

D5 (6.25 ng/ml):

Pipette 250 µl of D4 into 250 µl of Reference Standard & Sample Diluent

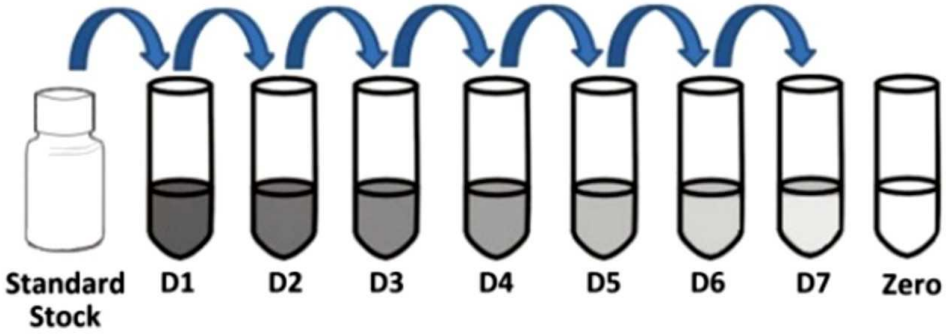
D6 (3.125 ng/ml):

Pipette 250 µl of D5 into 250 µl of Reference Standard & Sample Diluent

D7 (1.563 ng/ml):

Pipette 250 µl of D6 into 250 µl of Reference Standard & Sample Diluent

Zero Standard (0 ng/ml): Use Reference Standard & Sample Diluent alone



Wash Buffer: If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved. Prepare 750 ml of Working Wash Buffer by diluting the supplied 30 ml of 25x Wash Buffer Concentrate with 720 ml of deionized or distilled water. Wash Buffer can be stored at 4°C once prepared.

1x Biotinylated Detection Antibody: Calculate the required amount needed before beginning the experiment (50 µl/well) and include a 200µl excess. Centrifuge the stock tube before use. Dilute the concentrated Biotinylated Detection Antibody to the working concentration using the Detection Antibody Diluent (1:100).

1x HRP Conjugate: Calculate the required amount needed before beginning the experiment (100 µl/well) and include a 200 µL excess. Dilute the HRP Conjugate to the working concentration using the HRP Conjugate Diluent (1:100).

TMB Substrate: Using sterile techniques remove the needed volume of TMB Substrate Solution for the number of wells you are planning to run. Dispose of unused TMB Substrate Solution rather than returning it to the stock container.

Reagent Preparation Notes

1. It is highly recommended that standard curves and samples are run in duplicate within each experiment.
2. Once resuspended, standards should be used immediately, and used only once. Long-term storage of reconstituted standards is NOT recommended.

3. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
4. Do not prepare Standard dilutions directly in wells.
5. Prepared Reagents may adhere to the tube wall or cap during transport; centrifuge tubes briefly before opening.
6. All solutions should be gently mixed prior to use.
7. Reconstitute stock reagents in strict accordance with the instructions provided.
8. To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10μL is not recommended.
9. Substrate Solution is easily contaminated; sterility precautions should be taken. Substrate Solution should also be protected from light.
10. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
11. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

Assay Procedure

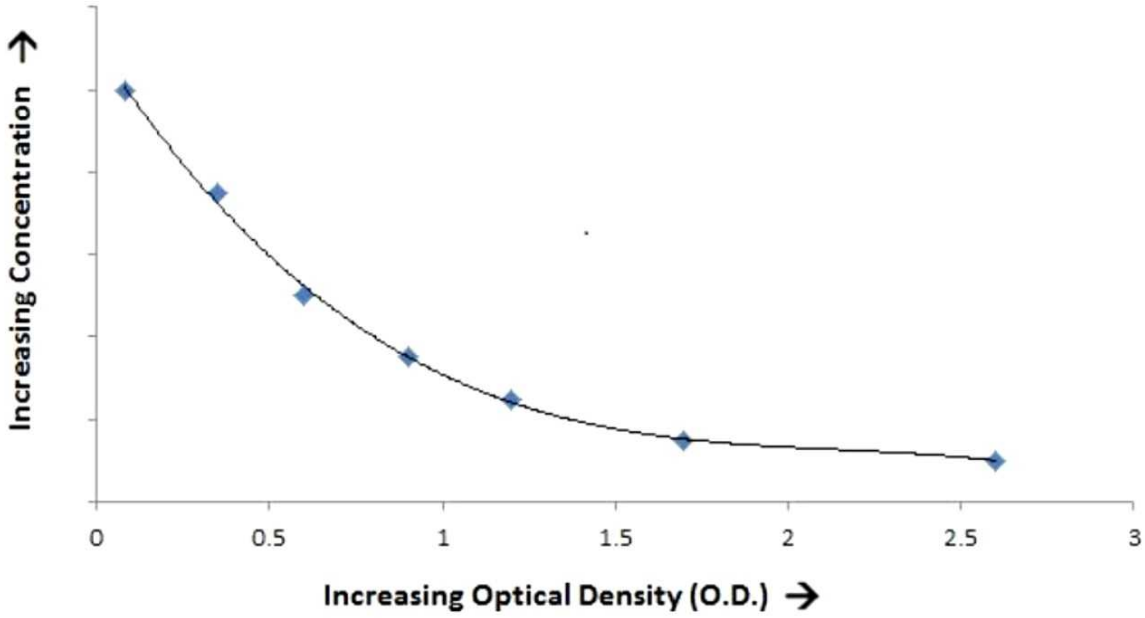
1. ELISA Plate: Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant and stored at -20°C.
2. Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. Assay Timing: The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. 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 of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. Washing: Proper washing 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 during the washing process. Do not put absorbent paper directly into the reaction wells.
7. Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance readings.
8. Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any 17 residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
9. Reaction Time Control: Control reaction time should be strictly followed as outlined.
10. Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.

11. Mixing: During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable results.
12. To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

Calculation

Average the duplicate readings for each standard, control, and sample. Create a standard curve by plotting the mean absorbance for each standard on the X-axis against the target antigen concentration on the Y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration on the Y-axis versus the O.D. of the standards on the Xaxis and the best fit line can be determined by regression analysis. The linear equation (Y = mx + b) can be used to calculate the standard curve where Y is the log of the concentration of the standard and x is the O.D. value of the standard. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data: The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



Specificity

N/A

Precautions

Before using this kit, researchers should consider the following:

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.

3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution.
7. Ensure that the kit is properly stored and do not use it beyond its expiration date.
8. When using multiple lots of the same kit do not substitute reagents from one kit to another. Review each manual carefully as changes can occur between lots. To control for inter-assay variability include a carry-over control sample.