



User's Manual

Vitamin B6 ELISA Kit



DEIASL336



96T



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

The Vitamin B6 ELISA Kit is used for quantitative analysis of Vitamin B6 in cereals (maize meal, soybean meal, millet flour, and rice flour), milk, milk powder and other food preparations.

General Description

Vitamin B6 refers to a group of chemically similar compounds which can be interconverted in biological systems. Vitamin B6 is part of the vitamin B group of essential nutrients. Its active form, pyridoxal 5'-phosphate, serves as a coenzyme in some 100 enzyme reactions in amino acid, glucose, and lipid metabolism.

Principles of Testing

The Vitamin B6 ELISA kit is based on indirect-competitive ELISA. The microtiter wells are coated with coupling antigen. Vitamin B6 in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used to show the color. Absorbance of the sample is inversely proportional to the Vitamin B6 concentration in it. After comparing with the standard curve, multiplied by the dilution factor, Vitamin B6 residue quantity in the sample can be calculated.

Reagents And Materials Provided

1. Anti-Vitamin B6 Coated Microtitre Plate (96 wells) – 1 no
2. Biotinylated Vitamin B6 Antibody – 1 ml
3. Standard (concentrated, 160 ng/ml) – 0.5 ml
4. Streptavidin-HRP Conjugate – 6 ml
5. (30X) Wash Buffer – 20 ml
6. Standard Diluent – 3 ml
7. TMB Substrate A – 6 ml
8. TMB Substrate B – 6 ml
9. Stop Solution – 6 ml
10. Instruction Manual

Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer

5. Timer
6. Absorbent Paper
7. Potassium Hexacyanoferrate(II)-3-hydrate (150 gm/l; Carrez I)
8. Zinc Sulfate-7-hydrate (300 gm/l; Carrez II)
9. Double-distilled water
10. 1M Caustic Soda solution
11. 1M Hydrochloric acid

Storage

1. All reagents should be stored at 2°C to 8°C for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Specimen Collection And Preparation

The vitamin is extracted from the sample by double-distilled water. After the dissolution, the pH is adjusted by 1 M caustic soda solution or 1 M hydrochloric acid to 6-7. Afterwards potential turbid matter is precipitated by Carrez I (150 gm/l Potassium Hexacyanoferrate(II)-3-hydrate) and Carrez II (300 g/l Zinc Sulfate-7-hydrate). The extract is filled up to a defined volume and is centrifuged. Samples which are difficult to dissolve in cold water can be brought in solution by gentle warming. After the centrifugation, the samples are further diluted by the supplied sample diluent. To exclude interfering matrix or pH effects, a minimal dilution of 1 in 5 should be followed. We recommend a dilution to 1-10 ng/ml, in order to obtain an optimal accuracy during the measurement.

Milk

Homogenized milk can be directly applied in the test. If required, dilute milk samples with sample buffer to increase sensitivity and avoid matrix interferences. Add 50 ul per well for the assay.

Milk Powder (other milk preparations)

Suspend 1 gm sample in 10 ml distilled water. (Dilution Factor = 1:10). Mix for 10 min. Heat the diluted sample for 3 min at 100°C (212 °F) in a water bath. Cool down quickly in an ice bath. Dilute the supernatant or filtrate with sample buffer to increase sensitivity and avoid matrix interferences. Add 50 ul per well for the assay.

Grain Products (Corn Flakes and Muesli)

Weight 3-5 grams of sample and homogenize by a mortar or a mixer, extracted by double-distilled water, the pH is adjusted to 6-7, and 0.5 mL each of Carrez I and Carrez II are added. Afterwards the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent. Grain products normally contain low concentrations of Vitamin B6. In order to avoid high dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water.

Grain Products (Bread and other preparations)

Grain products normally contain low concentrations of Vitamin B6. In order to avoid high dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water. The amount of sample diluent supplied in the kit is not sufficient in this case. The sample diluent may be ordered from Creative Diagnostics separately.

Multivitamin Juices

The juice is adjusted to pH 6-7, 0.5 mL each of Carrez I and Carrez II are added, and the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Multivitamin Jam

The jam is homogenised in a mixer, and approximately 8 grams are extracted by double-distilled water, the pH is adjusted to 6-7 and 0.5 mL each of Carrez I and Carrez II are added. Afterwards the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Multivitamin Sweets

The sweets are dissolved by gentle heating (if necessary) in double-distilled water, the pH is adjusted to 6-7, and 0.5 mL each of Carrez I and Carrez II are added. Afterwards the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent.

Multivitamin Tablets and Capsules

The tablets and capsules are dissolved in double-distilled water, and the pH value is adjusted to 6-7. Then 0.5 mL each of Carrez I and Carrez II are added, and the solution is filled up to a defined volume by double-distilled water. The solid matter is separated by centrifugation, and the upper phase is further diluted by sample diluent. To dissolve the capsules, heating to 30-40°C is recommended.

Reagent Preparation

Bring all reagents to Room Temperature prior to use.

To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

Assay Procedure

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Vitamin B6. High Dose Hook Effect is due to excess of antibody for very high concentrations of Vitamin B6 present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Vitamin B6 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in

under-estimation of the amount of Vitamin B6.

4. It is recommended that all Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples.

Assay Steps

- 1) Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples should be run in duplicates or triplicates. A standard curve is required for each assay.
- 2) Standards Dilution: Prepare the standards as per the table given below using the provided standard Concentration and standard diluent.

Standard Concentration	Standard No	Dilution Particulars
160 ng/ml	Standard, concentrated	Original Standard provided in the Kit*
80 ng/ml	Standard No.5	120 ul Original Standard + 120 ul Standard Diluent
40 ng/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
20 ng/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
10 ng/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
5 ng/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent

* refer accompanying sheet with the Standard, concentrated in the kit

- 3) The quantity of the plates depends on the quantities of samples and standards to be tested. It is suggested to remove the number of strips required for the assay.
- 4) Pipette out 50 ul of Standards and 40 ul Samples into the respective wells as mentioned in the work list. Note do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.
- 5) Pipette out 10 ul of Biotinylated Vitamin B6 Antibody into each sample well. Do not pipette into the blank and standards wells.
- 6) Pipette out 50 ul of Streptavidin:HRP Conjugate into each sample and standards well. Do not pipette into the Blank well.
- 7) Cover the plate and Incubate for 1 hour at 37°C in the incubator.
- 8) Aspirate and wash plate 4 times with 1X Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
- 9) Add Substrate A 50 ul and Substrate B 50 ul respectively to each well. Gently mix.
- 10) Cover the plate and Incubate for 10 min at 37°C in dark.
- 11) Pipette out 50 ul of Stop Solution. Wells should turn from blue to yellow in colour.
- 12) Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution blanking on the zero standards.

Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Calculation

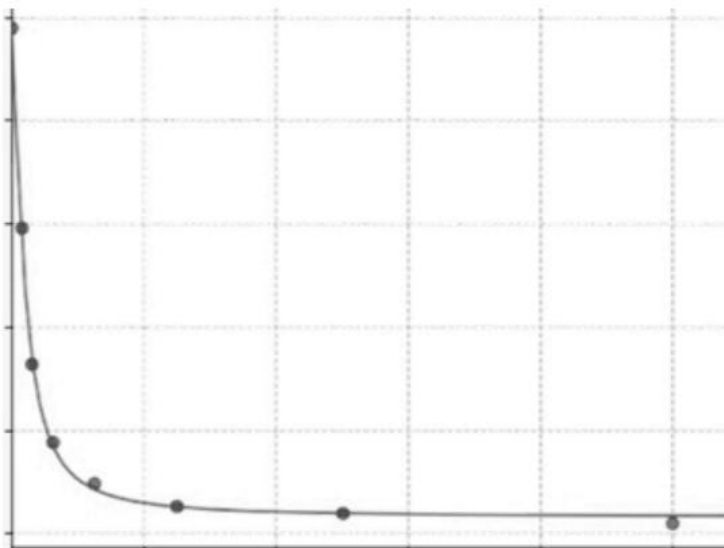
Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points.

To determine the unknown Vitamin B6 concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Vitamin B6 Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Typical Standard Curve

Typical Graph



Precision

Intra-Assay: CV<18%

Inter-Assay: CV<20%

Detection Range

5ng/ml - 80ng/ml

Detection Limit

It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be 2 ng/ml.

Specificity

The antibodies used in the kit for capture and detection are specific for Vitamin B6. The antibodies are monoclonal antibodies which are highly specific.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Vitamin B6 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8
pasteurized milk (n=3)	84-111%	83-117%	81-120%
cornflakes (n=3)	81-101%	81-118%	83-119%
bread (n=3)	82-109%	82-104%	81-117%
juice (n=3)	85-114%	81-111%	82-118%

Precautions

1. This Kit is For Research Use only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reconstituted reagents.
3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept at 2 - 8 °C before use in the original shipping container.
7. Some of the reagents contain small amounts (< 0.1 % w/w) sodium azide as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
8. Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
10. In any case GLP should be applied with all general and individual regulations to the use of this kit.

Limitations

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the

spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.