QUALICHEK™ Vitamin B9 (Folic Acid) ELISA

REF : KBFP1527

Ver 1.2

Enzyme Immunoassay for the Quantitative Determination of Vitamin B9 (Folic Acid) in Food Preparations.

NOT FOR DIAGNOSTIC OR HUMAN USE		**REF**	**Catalog Number**
	**Store At 2-8ºC**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KBFP1527** Σ **96 tests**

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Introduction:

Vitamin B9, also known as Folic Acid or Folic Acid e, it is a water-soluble vitamin. This vitamin is found in food, and manufactured as a dietary supplement and medication. Food sources of Folic Acid e include whole grains, legumes, and some meats and fish. Vitamins are part of many of the chemical reactions in the body. Folic Acid (Vitamin B9) helps the body's cells change carbohydrates into energy. The main role of carbohydrates is to provide energy for the body, especially the brain and nervous system. Folic Acid also plays a role in muscle contraction and conduction of nerve signals.

Intended Use:

The QUALICHEK™ Vitamin B9 ELISA Kit is used for quantitative analysis of Vitamin B9 in cereals (maize meal, soybean meal, millet flour, and rice flour), milk, milk powder and other food preparations.

Principle:

The QUALICHEK ™ Vitamin B9 ELISA kit is based on quantitative sandwich ELISA. Antibodies to Vitamin B9 are pre-coated onto microwells. Samples and standards are pipetted into microwells and Vitamin B9 present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-Vitamin B9 antibody is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Vitamin B9 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials provided in the Kit:

1. Vitamin B9 Coated Microtitre Plate (8 x 12 wells) - 1 no
2. Biotinylated Vitamin B9 Antibody - 1 ml
3. Standard (concentrated, 24 ng/ml) - 0.5 ml
4. Streptavidin-HRP Conjugate - 6 ml
5. (1X) Sample Diluent - 12 ml
6 (20X) Wash Buffer - 25 ml
7. Standard Diluent - 3 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual

Materials to be provided by the End-User:

1. Sample Diluent **available to purchase separately from KRISHGEN BIOSYSTEMS*
2. Microtiter Plate Reader able to measure absorbance at 450 nm.
3. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
4. Deionized (DI) water
5. Wash bottle or automated microplate washer
6. Timer
7. Absorbent Paper
8. Potassium Hexacyanoferrate(II)-3-hydrate (150 gm/l; Carrez I)
9. Zinc Sulfate-7-hydrate (300 gm/l; Carrez II)
10. Double-distilled water
11. 1M Caustic Soda solution
12. 1M Hydrochloric acid

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC 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 (18-25ºC). 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.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For In-Vitro Testing Only. Not for Diagnostic or Human Use.

Sample 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 sample diluent. To exclude interfering matrix or pH effects, a minimal dilution of 1 in 5 should be followed. We recommend a dilution to 50-100 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 interences. Add 40 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 interences. Add 40 ul per well for the assay.

Grain Products (Corn Flakes and Muesli)
3-5 grams of sample are homogenised 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 B9. 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 B9. In order to avoid high dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water. The sample diluent is not supplied in the kit and may be ordered from KRISHGEN BIOSYSTEMS 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.

Sample Dilution

Please note the kit is validated for use with neat samples.
Incase the user wishes to estimate the concentration of the target protein in the test sample, we advise to select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit and several trials may be necessary. If samples contain very high concentrations of the analyte, dilute the samples with the Sample Diluent provided in the kit.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25 ml of 20X Wash Buffer in 475 ml of DI water**.
4. **Standards Preparation**: Dilute 120 ul of original **Standard (96 ng/ml)** with 120 ul of standard diluent to generate a **48 ng/ml Standard stock solution**. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the **Standards** by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard No	Dilution Particulars
24 ng/ml	Standard, concentrated	Original Standard provided in the Kit*
12 ng/ml	Standard No.5	120 ul Original Standard + 120 ul Standard Diluent
6 ng/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
3 ng/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
1.5 ng/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
0.75 ng/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent
0 ng/ml	Standard No.0	50 ul Standard Diluent

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 B9. High Dose Hook Effect is due to excess of antibody for very high concentrations of Vitamin B9 present in the sample.
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 B9.
4. It is recommended that all Standards 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 Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50 ul Standard Diluent** to respective blank wells.

3. Add **50 ul prepared Standards** to respective standard wells.

4. Add **40 ul Samples** to respective sample wells.

5. Pipette **10 ul Biotinylated Vitamin B9 (Folic Acid) Antibody** to respective sample wells.
   Note: Do not add **Biotinylated Vitamin B9 (Folic Acid) Antibody** to standard and blank wells. The standards provided in the kit are pre-offered as a complex of the standard and the biotin antibody for ease-of-use.

6. Pipette **50 ul Streptavidin:HRP Conjugate** to all wells. Mix well.

7. Cover the plate with a sealer and incubate for **60 minutes at 37°C.**

8. Aspirate and wash plate 4 times with diluted **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

9. Pipette **100 ul TMB Substrate** to all wells

10. Incubate the plate at 37°C for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

11. Pipette **100 ul of Stop Solution** to all wells. The wells should turn from blue to yellow in color.

12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

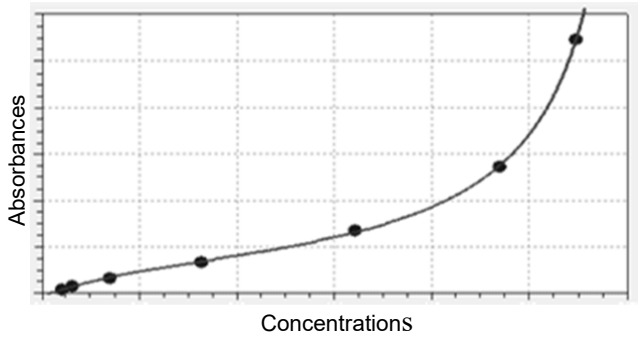
Calculation of Results:

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 B9 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 B9 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 Graph



Performance Characteristics:

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.

Sensitivity:
Limit Of Quantification: It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be **0.72 ng/ml.**

Standard Calibration Range:
0.75 ng/ml - 12 ng/ml

Specificity:
The antibodies used in this kit are monoclonal antibodies specific for Vitamin B9.

Precision:
Intra-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Vitamin B9 were tested in triplicate respectively. The Intra-Assay was found to be <15%
Inter-Assay Precision: 3 samples (n=3) with low, middle and high concentration of Vitamin B9 were tested in triplicate on two plates respectively on two consecutive days. The Inter-Assay was found to be <18%.
The Cumulative Variance % was calculated as CV (%) = SD/mean x 100 [SD=standard deviation]

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.

Safety Precautions:

- **This kit is For In-Vitro Test only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8°C before use in the original shipping container.
- 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.
- 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.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

Components (2-8ºC) → 30 mins RT Before Use → 

Thaw at Room Temperature (18-24ºC) → USE NOW

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

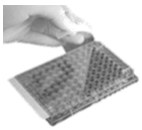
3.  Pipette **50 ul Standard Diluent** into respective blank wells.

4.  Pipette **50 ul prepared Standards** into respective Standard wells.

5.  Pipette **40 ul Samples** into the respective wells.

6.  Pipette **10 ul Biotinylated Antibody** to the sample wells only. Note: Do not add Biotinylated Antibody to standard well because the Standard Solution contains the biotinylated antibody.

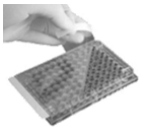
7.  Pipette **50 ul Streptavidin:HRP Conjugate** to all wells.

8. Cover plate  and incubate for 

at 37°C.

9.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

10.  Pipette **100 ul TMB Substrate** to all wells.

11. Cover plate  and incubate for 

at 37°C.

12.  Pipette **100 ul Stop Solution** in all wells.

13. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Zero Std Zero Std			
1B 2B	Standard No.1 Standard No.1			
1C 2C	Standard No.2 Standard No.2			
1D 2D	Standard No.3 Standard No.3			
1E 2E	Standard No.4 Standard No.4			
1F 2F	Standard No.5 Standard No.5			
1G 2G	Sample			
1H 2H	Sample			
3A 4A	Sample			
3B 4B	Sample			

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	Coated Microtiter Plate (8 x 12 wells)
STD	Standard
BIOTIN AB	Biotinylated Antibody
HRP CONJ	Conjugate Horseradish Peroxidase
STD DIL	Standard Diluent
1X SAMP DIL	(1X) Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature