Folic Acid ELISA Kit

Enzyme Immunoassay for the quantitative determination of Folic Acid in food

Catalog number: ARG80798

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Folates play an important role in the synthesis of nucleic acids and some amino acids and gained re-cently increased interest because they belong to the group of antioxidative vitamins. In the last years the in-fluence of folic acid supplementation to avoid abortion and dysraphism was a topic of research increasingly. Folic acid as the most stable representative of the group of folates is added to a broad range of food.

Traditional methods are mostly microbiological ones, but also TLC and HPLC are applied. These methods are time consuming and need complex equipment. This test kit allows the detection (2.5 to 4 hrs. incl. sample preparation) of folic acid in supplemented food which is more rapid compared to traditional techniques (24-48 hrs).

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. A folic acid conjugate is bound on the surface of a microtiter plate. Folic acid containing samples or standards and an antibody directed against folic acid are given into the wells of the microtiter plate. Immobilized and free folic acid compete for the antibody binding sites. After one hour incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugate against the antibody is given into the wells and after another hour incubation, the plate is washed again. Then a substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution,

and the color turns yellow. The yellow color is measured at 450 nm. The concentration of folic acid is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Folic acid conjugate-coated microplate	12 strips x 8-well	4°C
Anti-Folic Acid Antibody	6 ml (ready to use)	4°C
HRP-antibody Conjugate	15 ml (ready to use)	4°C
Standards (0, 4, 10, 40, 100, 400 ng/ml)	6 X 1 ml (ready to use)	4°C
Sample Diluent	2 X 60 ml	4°C
10x Wash Buffer	60 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer and Extraction Buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

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 g/L Potassiumhexacyanoferrate(II)-3-hydrate) and Carrez II (300 g/L Zincsulfate-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 10 should be followed. We recommend a dilution to 4-100 ng/ml, in order to obtain an optimal

accuracy during the measurement. Grain products normally contain low concentrations of folic acid. 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.

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.

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.

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 folic acid. In order to avoid high dilutions, the sample can be extracted directly by sample diluent instead of double-distilled water.

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.

Dry Milk Instant Formula

10 g of dry milk instant formula are suspended in 25 ml PBS and filled up to 50 ml. The mixture is vortexed intensely for 10 min and heated for 3 min in boiling water afterwards. After cooling to 20-25°C it is centrifuged for 10 min at 3000 g. The upper fat layer is aspirated and discarded. The remaining aqueous layer is diluted 1:5 in sample diluent.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells. Immediately add 50 µl folic acid antibody into each well.
3. Incubate for 60 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of HRP-Antibody Conjugate into each well. Incubate for 60 minutes at RT.
6. Aspirate and wash well as step 4.
7. Add 100 µl of TMB mixture to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

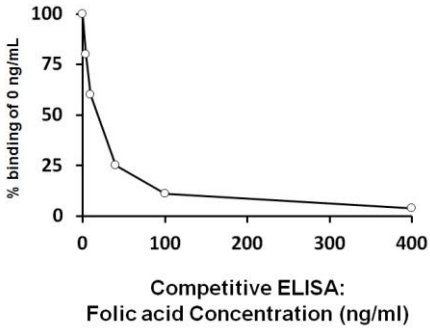
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The sensitivity of the Folic Acid is 2 ng/ml (based on the standard curve).

Specificity

For the following foods no cross-reactivity could be detected:

Cross-reactivity	Relative to folic acid (=100%)
Dihydrofolic acid	18%
Tetrahydrofolic acid	5%
5-Formyltetrahydrofolic acid	0.1%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3%

Recovery

The recovery of spiked samples was determined to 90-110 %.