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See external label	2°C-8°C	96 tests	REF 1736-8

Folic Acid

Cat # 1736-8

Enzyme Immunoassay for the quantitative determination of **Folic Acid** in food

Sensitivity	2 ng/mL
Recovery (spiked samples)	90-110%
Incubation Time	140 min

General Information

Folates play an important role in the synthesis of nucleic acids and some amino acids and gained recently increased interest because they belong to the group of antioxidative vitamins. In the last years the influence 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 Test

The **Diagnostic Automation Folic Acid** quantitative test is based on the principle of the enzyme linked immunosorbent assay. 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 for 20 minutes, resulting in the development of a blue colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of folic acid is indirectly proportional to the colour intensity of the test sample.

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25℃).
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5. Use a separate disposable tip for each specimen to prevent cross-contamination.
6. All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7. Do not mix components from different batches.
8. Do not use reagents after expiration date.
9. Check both precision and accuracy of the laboratory equipment used during the procedure (micro pipets, ELISA reader etc.).

Health and safety instructions

1. Do not smoke or eat or drink or pipet by mouth in the laboratory.
2. Wear disposable gloves whenever handling patient specimens.
3. Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4. Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

Reagents

The kit contains reagents for 96 determinations. They have to be stored at 2-8℃. Expiry data are found on the labels of the bottles and the outer package.

1. Microtiter plate consisting of 12 strips with 8 breakable wells each, coated with folic acid conjugate.
2. Folic Acid Standards (0; 4; 10; 40; 100; 400 ng/mL): 6 vials with 1.0 mL each, ready-to-use.
3. Anti-Folic Acid Antibody (mouse): 6 mL, dyed red, ready-to-use.
4. Conjugate (anti-mouse-IgG-HRP): 15 mL, dyed red, ready-to-use.
5. Substrate Solution (TMB): 15 mL; ready-to-use.
6. Stop Solution (0.5 M H_2SO_4): 15 mL; ready-to-use.
7. Sample Diluent (PBS): 2 x 60 mL, dyed red, ready-to-use.
8. Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate, dyed blue. Dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37℃ for 15 minutes.
9. Two plastic foils to cover the strips during the incubation.
10. Plastic bag to store unused microtiter strips.
11. Instruction Manual.

Additional Instrumentation and Reagents (not provided)

Instrumentation

- 10 - 1000 µL-micropipets
- ELISA reader (450 nm)
- Volumetric flask
- Mortar, mixer
- Centrifuge

Reagents

- Potassiumhexacyanoferrate(II)-3-hydrate (150 g/L; Carrez I)
- Zincsulfate-7-hydrate (300 g/L; Carrez II)
- Double-distilled water
- 1 M caustic soda solution
- 1 M hydrochloric acid

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 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. The buffer can however be ordered separately from **Diagnostic Automation**.

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

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 ℃ 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.

Procedure

1. Prepare samples as described above.
2. Pipet 100 µL standards or prepared samples in duplicate into the appropriate wells of the microtiter plate. Immediately add 50 µL folic acid antibody into each well.
3. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
4. Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition, empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.
5. Pipet 100 µL of conjugate (anti-mouse-IgG-HRP) into each well.
6. Cover the microtiter plate with a plastic foil and incubate for 60 minutes at room temperature on a microtiter plate shaker (or 90 minutes without shaker).
7. Wash the plate as outlined in 4.
8. Pipet 100 µL of substrate solution into each well.
9. Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10. Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue color will turn yellow upon addition.
11. After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The color is stable for 30 minutes.

Calculation of results

1. Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2. Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ng/mL on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis.
3. Using the mean optical density value for each sample, determine the corresponding concentration of

folic acid in ng/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

4. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

Example:

A vitamin tablet was extracted according to the described method. After Carrez precipitation the solution was filled up to 50 mL and the supernatant was diluted 1:500 with sample diluent. In the test a concentration of 40 ng/mL was determined. The resulting factor "F" is calculated as follows:

$$F = A \times B$$

A: Dilution factor 1 (in this case 50, since the tablet was dissolved in 50 mL)
B: Dilution factor 2 (in this case 500, since the supernatant was diluted after centrifugation 1:500)

The dilution factor has the dimension *mL/tablet*. The measured concentration is multiplied by the factor to get the real concentration.

Real concentration= 40 ng/mL x 25,000 mL/tablet = 1,000,000 ng/Tablette = 1 mg/tablet

Typical Standard Values

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 0 ng/mL standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

Folic acid (ng/mL)	(% binding of 0 ng/mL)
0	100
4	80
10	60
40	25
100	11
400	4

Performance

Sensitivity

The sensitivity of the **Diagnostic Automation Folic Acid ELISA** is 2 ng/mL (based on the standard curve).

Recovery

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

Intra-assay Precision

The intra-assay variation of the folic acid test was determined to 3%.

Cross-reactivity relative to folic acid (=100%)

Dihydrofolic acid	18%
Tetrahydrofolic acid	5%
5-Formyltetrahydrofolic acid	0.1%

References

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