Competitive enzyme immunoassay kit for quantitative analysis of Folic Acid

Catalog Number: EL2020-01

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

Folic acid, is one of the B vitamins. Folic acid is used to treat anemia caused by folic acid deficiency. It is also used as a supplement by women during pregnancy to prevent neural tube defects (NTDs) in the baby. Low levels in early pregnancy are believed to be the cause of more than half of babies born with neural tube defects. This kit is a new product based on ELISA, which is fast (only 45min in one operation), easy, accurate and sensitive compared with common instrumental analysis, and so it can considerably minimize operation error and work intensity.

2. Test Principle

This ELISA kit is designed to detect folic acid based on the principle of "**indirect-competitive**" enzyme immunoassay. The microtiter wells are coated with coupling antigen. Folic acid in the sample competes with the coating antigen for binding to the limited number of antibodies added. After the addition of a ready-to-use TMB substrate the signal is measured in an ELISA reader. The absorption is inversely proportional to the folic acid concentration in the sample.

3. Applications

This kit can be used in quantitative and qualitative analysis of folic acid residue in milk, milk powder, grain (rice, millet, maize, soybean, flour).

4. Cross Reactions

Folic acid.....100%

5. Equipment and Reagents Needed (not provided)

5.1 Equipment

- Microtiter plate spectrophotometer (450nm/630nm)
- Homogenizer / stomacher
- Shaker
- Vortex mixer
- Centrifuge
- Analytical balance (inductance: 0.01g)
- Graduated pipette: 10ml
- Rubber pipette bulb
- Volumetric flask: 100ml, 500ml
- Polystyrene Centrifuge tube: 2ml, 10ml
- Micropipettes: 20μl-200μl, 100μl-1000μl
- 250μl multi-pipette

5.2 Reagents

- Sodium chloride (AR)
- Deionized water

6. Components Provided in This Kit

- Microtiter plate with 96 wells coated with antigen
- Standard solutions (6 bottles x 1ml/bottle)
  0ppb, 1ppb, 3ppb, 9ppb, 27ppb, 81ppb
- Spiking standard solution (1ml/bottle)..... 10ppb
- Enzyme conjugate 7ml.....red cap
- Antibody solution 7ml.....green cap
- Solution A 7ml..... white cap
- Solution B 7ml .....red cap
- Stop solution 7ml.....yellow cap
- 20X Concentrated wash solution 40ml..... transparent cap
- 20X Concentrated Extraction solution 50ml.....blue cap

7. Reagents Preparation

Solution 1: 2% NaCl

- Dissolve 10g of sodium chloride with deionized water to 500ml, mix thoroughly

Solution 2: Extraction Solution

- Dilute the 2×concentrated extraction solution with deionized water in the volume ratio of 1:1(e.g. 10ml of 2×concentrated extraction solution + 10ml of deionized water), which will be used for dissolving the extracted sample. The diluted extraction solution can be conserved for 1 month at 4℃.

Solution 3: Wash solution

- Dilute the 20×concentrated wash solution with deionized water in the volume ratio of 1:19(e.g. 5ml of 2×concentrated wash solution + 95ml of deionized water), which will be used for dissolving the extracted sample. The diluted extraction solution can be conserved for 1 month at 4℃

8. Sample Preparations

8.1 Notice and precautions before operation:

- Please use one-off tips in the process of experiment and change the tips when absorbing different reagent.
- Make sure that all experimental tools are clean.
- Keep the untreated samples at 2-8°C.
- Use treated samples for assay immediately.

8.2 Milk

- Take 100μl of milk sample into a 2ml polystyrene centrifuge tube;
- Add 50μl of 2% NaCl (**Solution1**) and 850μl of extraction solution (**Solution 2**), vortex for 30 seconds to mix completely.
- Take 50μl of the prepared solution for assay.

Dilution factor.....10

8.2 Milk powder, grain (rice, millet, maize, soybean, flour)

- Take 0.5±0.05g of smashing milk sample or grain sample into a 10ml polystyrene centrifuge tube, add 5ml of 2% NaCl (**Solution1**), vortex for 2minutes to mix completely.
- Keep still for 5minutes.
- Transfer 100μl of the supernatant into a 2ml polystyrene centrifuge tube, add 900μl of extraction solution (**Solution 2**), vortex for 30 seconds to mix completely.
- Take 50μl of the prepared solution for assay.

Dilution factor.....100

9. Assay Steps

9.1 Instructions Prior to Beginning Assay

1. Ensure that all reagents and microwells are at room temperature (20-25℃).
2. Return all the rest reagents to 2-8℃ immediately after their use.
3. Wash the microwells correctly is important step in the process of the assay; this is a vital factor in the reproducibility of the ELISA analysis.
4. Avoid direct sunlight during the incubation; use the plate cover provided in the kit to cover the plate.

9.2 Steps in the Assay Process

1. Take all reagents out at room temperature (20-25°C) for more than 30minutes, homogenize before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. All reagents should be rewarmed before use.
4. **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. **Add standard solution / sample, enzyme conjugate and antibody:** Add 50μl of standard solution (**kit component**) or prepared sample to corresponding wells, then add 50μl of enzyme conjugate (**kit component**) and 50μl of antibody solution (**kit component**). Mix gently by shaking the plate manually and incubate for 30min 25℃ with cover.

6. **Wash:** Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 250µl of wash solution (**solution 3**) at interval of 10s for 5-6 times. Absorb the residual water with absorbent paper (*eliminate the air bubble with unused tips*).
7. **Coloration**: Add 50µl of solution A (**kit component**) and 50µl of solution B (**kit component**) to each well. Mix gently by shaking the plate manually and incubate for 15min at 25℃ with cover (see 12.8).
8. **Measure**: Add 50µl of the stop solution (**kit component**) to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm (*It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.*).

10. Results

10.1 Calculating the Percentage absorbance

- The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance (%) = B / B_0 *100%
B = absorbance standard (or sample)
B_0 = absorbance zero standard

10.2 Drawing a Standard Curve

- To draw a standard curve: take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the folic acid standards solution (ppb) as x-axis.
- The folic acid concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding Dilution factor of each sample followed, and the actual concentration of sample is obtained.

Please notice:

For data analysis of the ELISA kits, special software has been developed, which can be ordered on request.

11. Sensitivity, Accuracy and Precision

11.1 Test Sensitivity:

- 1ppb

11.2 Detection limit:

- Milk.....10ppb
- Milk Powder.....100ppb
- Grain.....100ppb

11.3 Accuracy:

- Milk.....90%±15%
- Milk Powder.....90%±15%
- Grain.....90%±15%

11.4 Precision:

- C.V. of the ELISA kit .....less than 10%

12. General Instructions

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.

12.3 Shake each reagent gently before use.

12.4 Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

12.5 Keep the ELISA kits at 2-8°C, do not freeze. Seal rest microwell plates Avoid straight sunlight for the standard sample and the colorless chromogenic reagent are sensitive to light.

12.6 Substrate solution should be abandoned if it turns colors. The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5(OD450nm<0.5).

12.7 The coloration reaction needs 20min after adding Solution A and Solution B. And you can prolong the incubation time to 25min if the color is too light to be determined. Never exceed 30min, On the contrary, shorten the incubation time properly.

12.8 The optimal reaction temperature is 25°C. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage Condition & Period

- Storage condition: 2-8℃.
- Storage period: 12 months

Who we are

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