FOR RESEARCH USE ONLY!

Folic Acid ELISA Kit

rev 01/19

(Catalog # E4523-100; 100 assays, Store kit at -20°C)

I. Introduction:

Folic acid (also called folate or vitamin B9) belongs to one of the antioxidative water-soluble B vitamins. Folic acid is an essential vitamin and human need to get it from the diet. It is naturally abundant in many foods but it is particularly enriched in dark green vegetables and liver. The main functions of folic acid are to synthesize nucleic acids and metabolize amino acids for cell division. Folic acid is also suggested to be critical for promoting fertility and preventing heart diseases. Folate deficiency may cause diarrhea, depression, confusion, anemia, and fetal neural tube defects. The traditional techniques/instruments (HPLC or GC-MS) for detecting folic acid are complex, expensive, laborious, and time-consuming. Immunoassay techniques, such as ELISA, are commonly preferred as a simple, reliable and rapid methods. BioVision's Folic Acid ELISA Kit is a competitive-based ELISA that detects folic acid in tissues, urine and serum. This detection kit offers ready-to-use reagents, and can quantify a broad range of folic acid (2 – 250 ng/ml) within 90 minutes.

II. Applications:

This ELISA kit is used for *in vitro* quantitative determination of folic acid
Detection Range: 2 - 250 ng/ml
Detection limit: 50 pg/well (1 ng/ml)
Cross Reactivity: Pefloxacin – 0%, Kanamycin – 0%

III. Sample Type:

Serum, urine and tissues (e.g. pork, liver, chicken, fish and shrimp)

IV. Kit Contents:

Components	E4350-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4523-100-1
Folic Acid Standard	2 vials	Yellow	E4523-100-2
HRP-conjugate Stock	25 µl	Blue	E4523-100-3
Antibody	7 ml	NM/Red	E4523-100-4
TMB substrate	12 ml	Amber	E4523-100-5
Stop Solution	10 ml	NM/Blue	E4523-100-6
Sample Diluent	20 ml	NM	E4523-100-7
Wash Buffer (10X)	50 ml	NM	E4523-100-8
Serum Solution	0.25 ml	Blue	E4523-100-9
Standard Buffer	40 ml	WM	E4523-100-10
Conjugate Buffer	7.5 ml	NM/Green	E4523-100-11
Plate Sealers	4	--	E4523-100-12

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 1 month at -20°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **TMB Substrate, Stop Solution and Sample Diluent:** Ready to be used. After use, store them at 4°C.
- **Serum Solution**: Ready to use. Bring bottle to room temperature before use. Store at -20°C.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **HRP-conjugate working solution**: Spin briefly before opening the tube. Pipet 20 µl of HRP-conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the conjugate solution bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Folic Acid Standard**: Add 1 ml of Standard Buffer into a Folic Acid Standard to prepare 25 µg/ml stock. Dilute the stock by 100 folds (e.g. 10 µl in 990 µl of buffer) to prepare the S5 standard (250 ng/ml) in below. Perform 2-fold dilution of S5 for S4 standard (eg. 400 µl in 400 µl of buffer). Perform 5-fold serial dilutions from S5 (e.g. 200 µl in 800 µl of buffer) to prepare S3 to S1 standards sequentially. S0 is the Standard Buffer only. These standards can be stored at -20°C for 2 weeks.

Standards	S0	S1	S2	S3	S4	S5
Concentrations (ng/ml)	0	2	10	50	125	250

VIII. Sample Preparation:

- **Serum**

1. Add 30 µl of Serum Solution into 270 µl of serum in an eppendorf tube and vortex well.

2. Incubate the sample at 37°C for 45 min.
3. Incubate the sample at 85-90°C for 10 min
4. Dilute the sample 10 fold with Sample Diluent. For example, mix 100 µl of treated serum with 900 µl of Sample Diluent.
5. Use 50 µl per well for the assay.
   Note: Dilution factor: 10

- **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and recover the supernatant.
2. Dilute the supernatant 10 fold with Sample Diluent. For example, mix 100 µl of urine with 900 µl of Sample Diluent.
3. Use 50 µl per well for the assay.
   Note: Dilution factor: 10

- **Tissue (pork, liver, chicken, fish and shrimp)**

1. Homogenize 0.1 g of tissue sample with 0.5 ml of Sample Diluent. Vortex for 5 min.
2. Centrifuge the sample at 10,000 x g, 4°C for 15 min and recover the supernatant.
3. Dilute the supernatant by 10 fold with Sample Diluent. For example, mix 100 µl of the supernatant with 900 µl of Sample Diluent
4. Use 50 µl per well for the assay.
   Note: Dilution factor: 10

IX. Folic Acid ELISA Assay Protocol:

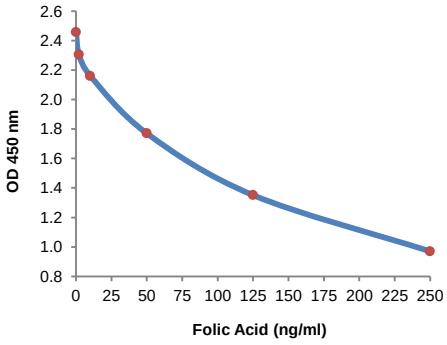
Notes: It is recommended that all standards and samples should be run at least in duplicate. Standard curves must be run each time the assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII respectively.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of conjugate working solution and 50 µl of Antibody to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 4 times: add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (This is essential for accurate results.) Repeat this step 3 more times. Remove the last wash by aspiration.
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no folic acid (S0). When its reading is approximately between 1.0 and 1.1 (usually between 15-30 min after adding the TMB Substrate), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm for the standards and samples within 10 min.

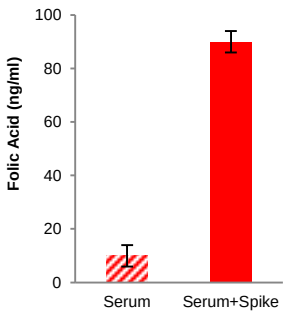
X. Calculation:

The Standard Curve is done by plotting the OD at 450 nm vs. folic acid concentration. The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration in the starting sample before dilution.

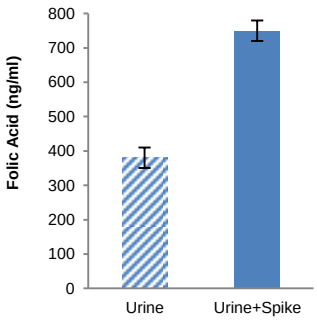
A.



B.



C.



Figures. **A.** Folic Acid standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B. C**. Spike recovery experiment: Human serum and urine samples were assayed with and without spike (100 and 800 ng/ml of folic acid total spike in serum and urine respectively) and showed 80-100% recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Enrofloxacin (ENR) ELISA Kit (Cat. No. E4277-100)
Fluoroquinolones ELISA Kit (Cat. No. 4205-100)
Kanamycin ELISA Kit (Cat. No. K4210-100)

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