QUALITY CONTROL:
We recommend that each laboratory uses Folate anemia controls to validate the performance of reagents.

REFERENCES:
1. "Serum and red blood cell folate concentrations for assessing folate status in populations." World Health Organization, Vitamin and Mineral Nutrition Information System, www.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng.pdf

Folate ELISA

Catalog No.: FA370A (96 Tests)

INTENDED USE
The Calbiotech, Inc. Folate ELISA test kit is intended for the quantitative determination of Folic acid in human serum and plasma. For Research Use Only. Not for use in Diagnostic Procedures.

PRINCIPLE OF THE TEST
The Folate test kit is a solid phase enzyme-linked immunoassay (ELISA), based on the principal of competitive binding. Streptavidin coated wells are co-incubated with extracted Folate standards, controls, samples, Folate Binding Protein-Biotin conjugate, and Folate-Enzyme conjugate at room temperature for 60 minutes. During the incubation, the folate-enzyme conjugate and the folate in the sample, standard, or quality control serum will compete to bind to the biotin-labeled folate binding protein. After the 60 minute incubation, all unbound conjugates are then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The color intensity is inversely proportional to the amount of Folate in the sample. The total assay procedure run time is 1.25 hours.

MATERIALS PROVIDED		96 Tests
1.	Microwell plate coated with Streptavidin	12x8x1
2.	Folate Standard Set: 6 vials	0.5 mL
3.	Biotinylated Folate Binding Protein Reagent, 1 bottle (ready to use)	7 mL
4.	Folate-Enzyme Conjugate, 1 bottle (ready to use)	7 mL
5.	Extraction Buffer, 1 bottle (ready to use)	8 mL
6.	Neutralization Buffer, 1 bottle (ready to use)	8 mL
7.	TCEP Solution, 1 bottle (40x)	0.25 mL
8.	Stop Solution, 1 bottle (ready to use)	12 mL
9.	TMB Substrate, 1 bottle (ready to use)	12 mL
10.	Wash Concentrate 20X, 1 bottle	25 mL

MATERIALS NOT PROVIDED
1. Precision pipettes
2. Disposable pipette tips
3. ELISA reader capable of reading absorbance at 450nm
4. Flat-head Vortex mixer
5. Plate shaker
6. Test tubes for sample preparation

Calbiotech Inc., | 1935 Cordell Ct., El Cajon, CA 92020

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The standards contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
2. This kit is intended For Research Use Only. Not for use in Diagnostic Procedures.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. Keep components of this kit protected from light and prolonged exposure to air.
6. It is recommended that standards, control and serum samples be run in duplicate
7. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION AND HANDLING

Serum, heparinized plasma or EDTA plasma samples can be used for the assay.

- For serum, collect whole blood by venipuncture and allow clotting.
- For plasma, mix the sample by gentle inversion prior to centrifugation.

Centrifuge and separate serum or plasma as soon as possible after collection. Do not use hemolyzed samples.
The specimens may be refrigerated at 2-8°C for two weeks. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed before analysis.

REAGENT PREPARATION

Before running the test, prepare the following:

1. **Extraction Agent:**
   Immediately before use, dilute an aliquot of the TCEP solution 1:40 with the extraction buffer. For example, to make 1 mL of extraction agent, add 25 uL of TCEP solution to 975 uL of extraction buffer.
2. **Sample Extraction:**
   Label enough test tubes for each of the standards, controls, and samples. Add 50 uL of each standard, control, and sample to be tested to individual tubes. Once all the standards, controls, and samples have been added, pipette 25 uL of extraction reagent to each sample. Vortex test tube after each addition. Allow the extraction to proceed for 15 minutes. After 15 min, pipette 25 uL of neutralization buffer to each standard, control, and sample. Vortex test tube after each addition. Allow the standards, controls, and samples to stand for 5 minutes to ensure complete neutralization**. Double all volumes for standards, controls, and samples to be run as replicates**.
   NOTE: Consistent extraction times are critical for consistent assay results.
3. **Prepare 1X Wash Buffer** by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

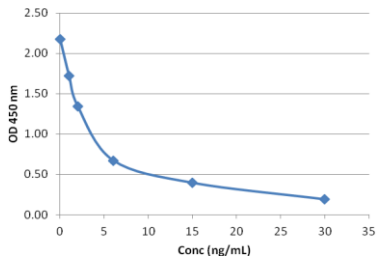
PROCEDURE:

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be GENTLY mixed without foaming. Once the procedure has started, all steps should be completed without interruption.

1. Place the desired number of coated strips into the holder.
2. Dispense 50µl of extracted Folate Standards, controls, and samples into appropriate wells.,
3. Dispense 50µl of enzyme conjugate, into each well.
4. Dispense 50µl of biotinylation folate binding protein conjugate into each well. Shake the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 60 minutes, at room temperature (20-25°C).
6. Briskly shake out the contents of the wells. Rinse the wells 3 times with 1X wash buffer. Strike the wells sharply on absorbent paper to remove residual water droplets.
7. Using a multi-channel pipette, dispense 100 µl of TMB Substrate into each well.
8. Incubate for 15 minutes at room temperature, preferably in the dark.
9. Add 50µl of stop solution to each well and gently mix until a uniform color, in each well, is obtained.
10. Read the absorbance in each well at 450nm within 15 minutes after adding the stop solution.

Standard Curve:

Six standard levels are included for each run. A typical standard curve is shown below.



Folate, (ng/ml)	Absorbance (450nm)
0	2.17
1	1.72
2.5	1.34
5	0.67
10	0.39
25	0.19