Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

# Folate Test System
# Product Code: 7525-300

## 1.0 INTRODUCTION

**Intended Use: The Quantitative Determination of Folate Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric**

## 2.0 SUMMARY AND EXPLANATION OF THE TEST

Folate supplementation has escalated over recent years with the knowledge of its many benefits. As one of the B vitamins, folate, or Vitamin B9, is involved in many bodily functions and deficiency can cause disease in not only the elderly, but infants too. Folate deficiency is associated with megaloblastic anemia, neural tube defects, and cardiovascular dieases.[1,2,3]

Folate plays an important role in brain development and therefore is vital during growth. The most common defects resultant from folate deficiencies are neural tube defects. With a vital role in nucleic acid synthesis, folate has been found to be beneficial as supplementation during pregnancy and other times of rapid tissue growth. Folate also plays a vital role in maintaining proper balance of homocysteine, a contributing factor in occurrences of occlusive vascular diseases and stroke. Individuals with susceptibility to heart disease and several forms of cancer may also benefit from supplementation. [1,2,4]

Major sources of folate include green leafy vegetables, legumes, beans and fortified cereals. Foods fortified with folate are actually fortified with folic acid because of the higher bioavailability for absorption by the body. In circulation, folate is present in several different forms, some of which are more stable than others. Folic acid and N-methyltetrahydofolate are two common forms, the latter being more stable and found in higher concentrations in serum. Due to the stability of the molecule, methytetrahydrofolate is very often used as the form focused on during methods of analysis.[4,5]

Folate binding proteins are responsible for folate metabolism. Two types exist in circulation: one type aids in binding to the cell surface and the other soluble form exists in circulation. These folate binding proteins also have the capability of binding several different folate derivatives including folic acid and N-methytetrahydrofolate. The interaction between folic acid and folate binding protein is greater than methyltetrahydrofolate. Current assays on the market require an extraction step to release the folate derivatives from the folate binding protein.[5,6]

In the past, folate has been quantified in samples using such methods as microbiological assays, bio-specific procedures and HPLC-MS techniques. Overall, this rapid rise in knowledge of folate, its importance, and subsequently folate supplementation has caused a higher demand for improved testing methods.[4]

## 3.0 PRINCIPLE

**Competitive Binding Protein Assay (TYPE 8):**
The essential reagents required for a competitive binding assay include specific binding protein, enzyme-antigen conjugate and native antigen. Upon mixing enzyme-antigen conjugate, biotinylated binding protein and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + BP_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgBP_{Btn} + {}^{Enz}AgBP_{Btn}$$

$BP_{Btn}$ = Biotinylated Binding Protein (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

$^{Enz}Ag$ = Enzyme-antigen Conjugate (Constant Quantity)

$BP_{Btn}$ = Antigen-Binding Protein Complex

$^{Enz}AgBP_{Btn}$= Enzyme-Antigen-Binding Protein Complex

$k_a$ = Rate Constant of Association

$k_{-a}$ = Rate Constant of Disassociation

$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the binding protein and the streptavidin immobilized on the microwell occurs. This effects the separation of the binding protein enzyme bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgBP_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$

$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
$\underline{Immobilized\ complex}$ = sandwich complex bound to the solid surface
The enzyme activity in the protein binding protein bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

## 4.0 REAGENTS

**Materials Provided:**

**A. Folate Calibrators – 1.0 ml/vial - Icons A-F**
Six (6) vials containing human serum albumin reference for Folate at concentrations of 0 (**A**), 1.0 (**B**), 2.5 (**C**), 5.0 (**D**), 10.0 (**E**), and 25.0 (**F**) in ng/ml. A preservative has been added. Store at 2-8°C.
**Note**: The calibrators, human serum based, were calibrated using a highly purified N-methyltetrahydrofolate preparation.

**B. Folate Enzyme Reagent – 7.0 ml/vial – Icon** E
One (1) vial containing Folate (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with dye. Store at 2-8°C.

**C. Folate Biotin Reagent – 7.0 ml/vial - Icon** ∇
One (1) vial containing biotinylated purified folate binding protein conjugate in buffer, dye and preservative. Store at 2-8°C.

**D. Streptavidin Coated Plate – 96 wells –Icon** ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

**E. Wash Solution Concentrate – 20.0 ml/vial - Icon**
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

**F. Substrate Reagent – 12.0 ml/vial - Icon** $S^N$
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide ($H_2O_2$) in buffer. Store at 2-8°C.

**G. Stop Solution – 8.0 ml/vial - Icon** STOP
One (1) vial containing a strong acid (0.5M $H_2SO_4$). Store at 2-8°C.

**H. Releasing Agent – 14.0 ml/vial - Icon** Ξ
One (1) vial containing a strong base (sodium hydroxide) and potassium cyanide. Store 2-8°C.

**I. Stabilizing Agent – 0.7 ml/ vial - Icon** Π
One (1) vial containing tris (2-carboxyethyl) phosphine (TCEP) solution. Store at 2-8°C.

**J. Neutralizing Buffer – 7.0 ml/ vial - Icon** NZ
One (1) vial containing buffer with dye that reduces the pH of sample extraction. Store at 2-8°C.

**K. Product Insert**

**Note 1:** Do not use reagents beyond the kit expiration date.
**Note 2:** Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
**Note 3:** Above reagents are for a single 96-well microplate**.**

**4.1 Required But Not Provided:**
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Glass test tubes for calibrator, control, and patient sample preparation.
5. Microplate washer or a squeeze bottle (optional).
6. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate covers for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

## 5.0 PRECAUTIONS

**For In Vitro Diagnostic Use**
**Not for Internal or External Use in Humans or Animals**

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

**Safe Disposal of kit components must be according to local regulatory and statutory requirements.**

## 6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of two (2) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 7 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

## 7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## 8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at room temperature (2-30°C) for up to 60 days.
2. **EXTRACTION AGENT**
Add an aliquot of the stabilizing agent in order to prepare a 1/40 (stabilizing agent / releasing agent) dilute solution. For example, to make 4ml (4000µl), add 0.100ml (100µl) stabilizing agent to 3.9ml (3900µl) releasing agent.
3. **SAMPLE EXTRACTION (*See Note 3)***
Obtain enough test tubes for preparation of all patient samples, controls, and calibrators. Dispense 0.10ml (100µl) of all samples into individual test tubes. Pipette 0.050ml (50µl) of the prepared extraction agent to each test tube, shaking *(see note 3)* after each addition. Let the reaction proceed for 15 min. At end of the 15 min, dispense 0.050 ml (50µl) of the neutralizing buffer, vortex *(see note 3)*. After the neutralization buffer is added and mixed, let the reaction go to completion by waiting an additional 5 min before dispensing into the microwells.

***Note 1: Do not use the working substrate if it looks blue.***
***Note 2: Do not use reagents that are contaminated or have bacteria growth.***
***Note 3: Use of multiple (3) touch vortex is recommended.***
***Note 4: It is extremely important to accurately dispense the correct volume with a calibrated pipette and by adding near the bottom of the glass tubes at an angle while touching the side of the tubes.***

## 9.0 TEST PROCEDURE

*Before proceeding with the assay, bring all reagents, calibrators and controls to room temperature (20 - 27°C).*
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Prepare all samples according to the "Sample Extraction" procedure in section "8.0 Reagent Preparation"; **it is important to wait 5 min before proceeding to allow the neutralization reaction to go to completion (see above).**
2. Format the microplates' wells for each calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
3. Pipette 0.050 ml (50 µL) of the appropriate extracted folate calibrator, control or specimen into the assigned well.
4. Add 0.050 ml (50 µl) of Folate Enzyme Reagent to all wells
5. Mix the microplate gently for 20-30 seconds.
6. Add 0.050 ml (50 µl) of the Folate Biotin Reagent to all wells.
7. Mix the microplate gently for 20-30 seconds.
8. Cover and incubate for 45 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
11. Add 0.100 ml (100 µl) of substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

**DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION**

12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within thirty (30) minutes of adding the stop solution.**

**Note**: Dilute the samples suspected of concentrations higher than 25ng/ml 1:5 and Folate '0' ng/ml calibrator and re-assay.

## 10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Folate in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate calibrator versus the corresponding Folate concentration in ng/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Folate for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.021) intersects the dose response curve at 11.9 ng/ml Folate concentration (See Figure 1).

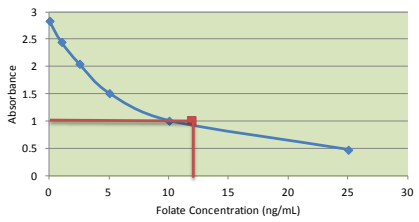
**Note:** Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*The data, figure and table below are for example only. Do not use it for calculating your results.

**EXAMPLE 1**

| Sample I.D. | Well Number | Abs (A) | Mean Abs (B) | Value (ng/ml) |
|---|---|---|---|---|
| Cal A | A1 | 2.812 | 2.839 | 0 |
| | B1 | 2.865 | | |
| Cal B | C1 | 2.437 | 2.455 | 1 |
| | D1 | 2.473 | | |
| Cal C | E1 | 2.058 | 2.055 | 2.5 |
| | F1 | 2.051 | | |
| Cal D | G1 | 1.542 | 1.518 | 5 |
| | H1 | 1.494 | | |
| Cal E | A2 | 1.003 | 1.015 | 10 |
| | B2 | 1.027 | | |
| Cal F | C2 | 0.453 | 0.485 | 25 |
| | D2 | 0.516 | | |
| Sample | E2 | 1.004 | 1.021 | 11.9 |
| | F2 | 1.038 | | |

Figure 1: Folate




## 11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

## 12.0 RISK ANALYSIS

*The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.*

### 12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

### 12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' *Clin.Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

## 13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" population the expected ranges for the Folate AccuBind® ELISA Test System are detailed in Table 1.

**TABLE 1**
**Expected Values for the Folate Test[7]**

| |
|---|
| Normal Adult Population > 3.0 ng/ml |

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## 14.0 PERFORMANCE CHARACTERISTICS

### 14.1 Precision

The within and between assay precision of the Folate AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**
Within Assay Precision (Values in ng/ml)

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Level 1 | 24 | 3.72 | 0.31 | 8.3 |
| Level 2 | 24 | 9.26 | 0.53 | 5.7 |
| Level 3 | 24 | 13.71 | 0.83 | 6.1 |

**TABLE 3**
Between Assay Precision (Values in ng/ml)

| Sample | N | X | σ | C.V. |
|---|---|---|---|---|
| Level 1 | 12 | 3.32 | 0.32 | 9.6 |
| Level 2 | 12 | 8.85 | 0.68 | 7.7 |
| Level 3 | 12 | 12.85 | 1.15 | 8.9 |

*As measured in ten experiments in duplicate over a ten day period.

### 14.2 Sensitivity

The Folate AccuBind® ELISA Test System has a sensitivity of 0.52ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

### 14.3 Accuracy

The Folate AccuBind® ELISA Test System was compared with a reference method. Biological specimens were used with values that ranged from 3.2ng/ml – 13.7ng/ml. The total number of such specimens was 30. The least square regression equation and the correlation coefficient were computed for this Folate ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

**TABLE 4**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| This Method (Y) | 7.76 | Y= 0.162 +1.07(X) | 0.984 |
| Reference (X) | 8.46 | | |

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

### 14.4 Specificity

The specificity of the Folate Binding Protein used to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations.

| Substance | Interference |
|---|---|
| Bilirubin | ND* |
| Biotin | ND* |
| Lipemia | ND* |

**ND=Not Detectable*

## 15.0 REFERENCES


1. Scholl, TO. *The American Journal of Clinical Nutrition.* 1996, 63, 520-525.
2. Clarke, R. *Age and Ageing.* 2004, 33, 34-41.
3. Owen, WE. *American Journal of Clinical Pathology.* 2003, 120, 121-126.
4. Arcot, J. *Trends in Food Science and Technology.* 2005, 16, 253-266.
5. Mantovani, LT. *European Journal of Cancer.* 1994, 30, 363-369.
6. Nygren-Babol, L. *International Dairy Journal.* 2009, 19, 437-442.
7. Snow, CF, M.D. *Archives of Internal Medicine.* 1999, 159, 1289-1298.



**Rev. 1** **Date: 2014-JUL-21** **DCO: 1003**
**Product Code: 7525-300**


| Size | | 96(A) | 192(B) |
|---|---|---|---|
| Reagent (fill) | A) | 1.0 ml set | 1.0 ml set |
| | B) | 1 (7 ml) | 2 (7 ml) |
| | C) | 1 (7 ml) | 2 (7 ml) |
| | D) | 1 plate | 2 plates |
| | E) | 1 (20 ml) | 2 (20 ml) |
| | F) | 1 (12 ml) | 2 (12 ml) |
| | G) | 1 (8 ml) | 2 (8 ml) |
| | H) | 1 (14 ml) | 2 (14 ml) |
| | I) | 1 (0.7 ml) | 2 (0.7 ml) |
| | J) | 1 (7 ml) | 2 (7 ml) |

For Orders and Inquiries, please contact


**Monobind Inc.**
100 North Pointe Drive
Lake Forest, CA 92630 USA
Tel: +1 949.951.2665 Email: info@monobind.com
Fax: +1 949.951.3539 Web: www.monobind.com


**Please visit our website to learn more about our other interesting products and services.**

IVD CE 2° 8°

EC REP **CEpartner4U**, Esdoornlaan 13, 3951DB Maarn, The Netherlands www.cepartner4u.eu

Meridian Healthcare®


**Meridian Healthcare srl**
Tel. +39 095 725 68 69 Fax:. +39 095 725 44 54
info@meridianhealthcare.it
www.meridianhealthcare.it