FOR REFERENCE USE ONLY

EDI™ Folate ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Determination of Folate (Vitamin B-9) Levels in Human Plasma or Serum Samples.

KT- 717

INTENDED USE
This ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of Folate (Vitamin B-9) levels in human plasma or serum samples. This kit is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY
Folate, sometimes specifically known as folic acid, folacin, or vitamin B-9, is a B Vitamin found naturally in grains or synthesized as vitamin supplements. It is classified as an essential vitamin, as it cannot be synthesized in the body but is required for normal physiological functions, including the production and maintenance of cells[2]. Folate deficiencies can result in a host of health effects ranging from heart disease, stroke, age-related macular degeneration, neurological deficiencies, and more[1].

ASSAY PRINCIPLE
This ELISA is designed, developed and produced for the quantitative measurement of Folate in human plasma or serum samples. Assay calibrators and patient samples are added directly to wells of a microplate that is coated with a Folate-binding protein, along with HRP-conjugated Folate. After the first incubation period, the binding protein on the wall of the microtiter well captures sample Folate and HRP-conjugated Folate in a competitive nature. Excessive amounts of unbound proteins and HRP-conjugated Folate in each microtiter well are washed away. For the detection of the captured sample Folate, the well is incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the complex bound to the Folate on the wall of the microtiter well is directly proportional to the amount of Folate in the sample. A calibrator standard curve is generated by plotting the absorbance versus the Folate concentration for each calibrator on point-to-point or cubical scales or 4 parameter curve fits. The concentration of Folate in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage
This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. **Folate Binding Protein Coated Microplate (31191)**
One microplate with 12 x eight strips (96 wells total) coated with a Folate binding protein. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **HRP Conjugated Folate (31192)**
One vial containing 12 mL of ready-to-use Folate analog labeled with HRP enzyme in protein buffer with stabilizers and dye. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **Releasing Buffer Concentrate (40X) (31185)**
One vial containing 0.5 mL of 40 times concentrated solution with releasing agents in buffer. It should be only used for 1X releasing buffer according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **Releasing Buffer Diluent (31184)**
One vial containing 12 mL solution with strong bases and potassium cyanide. It should be only used for releasing buffer dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **Neutralization Buffer (31186)**
One vial containing 7.0 mL ready-to-use buffer to reduce pH. It should be only used for sample extraction according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. **Wash Concentrate (40X) (31188)**
One bottle containing 25 mL of 40-fold concentrate. The contents must be diluted with 975 mL of demineralized water and mixed well before use. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with preservatives. The diluted buffer should be stored at room temperature and is stable until the expiration date on the kit box.

7. **ELISA HRP Substrate (10020)**
One bottle containing 12 mL of ready-to-use tetramethylbenzidine (TMB) with stabilized hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

8. **ELISA Stop Solution (10030)**
One bottle containing 8 mL ready-to-use solution of a strong acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

9. **Folate Calibrators (31200 - 31205)**
Six vials, each containing ready-to-use human serum based calibrators with preservatives. **Refer to vials for exact concentration for each calibrator.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

10. **Folate Control (31210)**
One vial containing ready-to-use human serum based control with preservatives. **Refer to vial for exact concentration.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

SAFETY PRECAUTIONS
The reagents must be used in a professional laboratory environment and are for in research use. The source material for reagents

containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 25 µL, 50 µL, 100 µL, and 1000 µL etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION

Only 100 µL of human plasma or serum is required for Folate measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected with Vacutainer. Separate the plasma from cells by centrifugation (850 – 1500xg for 10 minutes). The plasma should be separated from the cells within one hour of blood collection and transferred to a clean test tube. **Plasma samples should be stored at –20ºC** if the assay is not to be performed within 72 hours. Otherwise, the plasma samples should be stored at room temperature for up to 72 hours. It is important that the plasma samples must not be stored at 2 – 8ºC in any circumstance.

ASSAY PROCEDURE

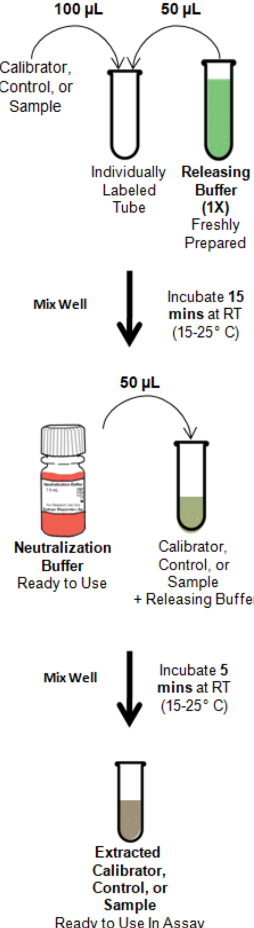
1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature(15-25° C). Reagents from different kit lot numbers should not be combined or interchanged.

(2) Wash Concentrate (40X) (31188) must be diluted to working solution prior to use. Please see REAGENTS section for details.

(3) Prepare 1x Releasing Buffer by 1:40 fold dilution of the Releasing Buffer Concentrate (31185) with the Releasing Buffer Diluent (31184). For each strip, mix 0.5 mL of the Releasing Buffer Diluent with 12.5 µL of Releasing Buffer Concentrate. **This 1x Releasing Buffer must be freshly prepared right before sample extraction.**

(4) **Sample Extraction Procedure:** Add 100 µL of the calibrators, control, and patient samples into appropriately labeled individual tubes. Add 50 µL of freshly prepared Releasing Buffer to each tube. Mix well by inversions or gentle vortexing and incubate static at room temperature (15-25° C) for 15 minutes. Add 50 µL of Neutralization Buffer (31186) to all tubes. Mix well by inversions or gentle vortexing and incubate static at room temperature (15-25° C) for 5 minutes.



2. Assay Procedure

(1) Place a sufficient number of Folate-binding protein coated microplate strips (31191) in a holder to run Folate calibrators and patient samples in duplicate.

(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	CAL 1	CAL 5	SAMPLE 2
B	CAL 1	CAL 5	SAMPLE 2
C	CAL 2	CAL 6	SAMPLE 3
D	CAL 2	CAL 6	SAMPLE 3
E	CAL 3	CTRL 1	SAMPLE 4
F	CAL 3	CTRL 1	SAMPLE 4
G	CAL 4	SAMPLE 1	SAMPLE 5
H	CAL 4	SAMPLE 1	SAMPLE 5

(3) Add 50 µL of freshly extracted calibrators, control and patient samples into the designated microwells.

(4) Add 100 µL of HRP Conjugated Folate Tracer (31192) to each microwell.

(5) Mix gently and cover the plate with one plate sealer and aluminum foil to avoid exposure to light. Incubate at room temperature (15-25° C) for **1 hour**.

(6) Remove aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 3 times by dispensing 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

(7) Add 100 µL of ELISA HRP substrate (10020) to each microwell.
(8) Mix gently and cover the plate with one plate sealer and aluminum foil to avoid exposure to light. Incubate at room temperature (15-25° C) for **15 minutes**.
(9) Remove the aluminum foil and plate sealer. Add 50 µL of Stop Solution (10030) into each of the microwells. Mix gently.
(10) Read the absorbance at 450 nm within 10 minutes in a microplate reader.
NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm, 620 nm or 630 nm.

PROCEDURAL NOTES

1. The 1x Releasing Buffer must be freshly prepared right before sample extraction. All calibrators, control and unknown samples must be freshly extracted according to the procedure.
2. It is recommended that all calibrators, control and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
3. Keep light-sensitive reagents in the original amber bottles.
4. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
5. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
6. Incubation times or temperatures other than those stated in this insert may affect the results.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

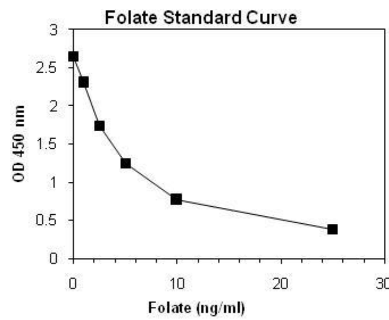
INTERPRETATION OF RESULTS

The Folate concentrations for the controls and patient samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from Folate ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450 nm Absorbance		
	Readings	Average	B/B0%
0 ng/mL	2.712 2.788	2.750	100
1 ng/mL	1.951 1.999	1.975	72
2.5 ng/mL	1.592 1.646	1.619	59
5 ng/mL	1.213 1.059	1.136	41
10 ng/mL	1.047 0.946	0.996	36
25 ng/mL	0.522 0.522	0.537	20



EXPECTED VALUES

Seventy-five (n =75) normal adult sera were measured with this Folate ELISA. The normal range was found to be greater than or equal to 3.0 ng/mL.

It is highly recommended that each laboratory should establish its own normal cut-off level.

LIMITATION OF THE PROCEDURE

1. For sample values reading greater than the highest standard or 90% value of the highest standard, it is recommended to re-assay samples with dilution by 1:4 and 1:10.
2. Serum samples are not as stable as plasma samples. Therefore, it is strongly recommended to use plasma sample for Folate measurement.
3. Bacterial or fungal contamination of plasma specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known Folate levels. We recommend that all assays include the laboratory's own Folate controls.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

1. "Folic Acid". Drugs.com. American Society of Health-System Pharmacists. 1 January 2010.
Pommerville, Glendale Community College Jeffrey C. (2009). Alcamo's Fundamentals of Microbiology: Body Systems. Jones & Bartlett Publishers. p. 511. ISBN 9780763787127

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

Short Assay Procedure of Folate

1. Add **50 µL** of calibrators, control and patient samples

   into the designated microwell.
2. Add **100 µL** of HRP Conjugated Folate to each well.
3. Mix, cover and incubate the plate at room for **1 hour**.
4. Wash each well 3 times.
5. Add **100 µL** of ELISA HRP substrate into each well.
6. Mix, cover and incubate the plate at room for **15 minutes**
7. Add **50 µL** of Stop Solution into each of the wells.
8. Read the absorbance at OD 450 nm.

Manufacturer	No. of tests
REF Catalog Number	LOT Lot Number
CONC Concentrate	Store at
IVD In Vitro Diagnostic Device	Use by
Read instructions before use	LOT Lot Number
Corrosive substances	EC REP Authorized Representative In Europe

Epitope Diagnostics, Inc.
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San Diego, CA 92121, USA

MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany