Product Manual

Folic Acid ELISA Kit

Catalog Number

MET-5068	96 assays
MET-5068-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Folic acid is a B vitamin also known as Vitamin B9. Since humans don't synthesize folic acid, it is required from the diet and is therefore considered to be an essential vitamin. In cells, folic acid is required for amino acid metabolism as well as to carry one-carbon groups used for methylation reactions and synthesis of nucleic acids (such as thymine and purine bases). Therefore, deficiency in folic acid disrupts DNA synthesis and cell division, affecting mostly hematopoietic cells and abnormal tissue growths because of their higher rate of cell division.

Folic acid is used to supplement folic acid deficiency. This deficiency can cause anemia. Symptoms of anemia can include fatigue, heart palpitations, difficulty breathing, open sores observed on the tongue, and color changes of the hair or skin. Deficiency can occur in children after only a month of consuming a folic acid deficient diet. In adults, normal total body folic acid levels are between 10,000–30,000 micrograms (µg) with plasma levels of greater than 7 nM (3 ng/mL) (Table 1). Women also take supplemental folic acid during pregnancy to prevent fetal neural tube defects (NTDs). Insufficient levels of dietary folic acid in early pregnancy are thought to be the cause of over half of babies born with neural tube defects. As a result, over 50 countries add folic acid to certain foods as a way to decrease NTD incidents in the population.

	Concentration (ng/mL)	Concentration (nM)
Adults	3-20	7-45.3
Children	5-21	11.3-47.6
Infants	14-51	31.7-115.5

Table 1. Reference ranges for folic acid in human plasma.

The Folic Acid ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of folic acid in serum, cell or tissue samples. The quantity of folic acid in unknown samples is determined by comparing its absorbance with that of a known folic acid standard curve. The kit has detection sensitivity limit of 24 pg/mL folic acid. Each Folic Acid ELISA Kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

The Folic Acid ELISA kit is a competitive ELISA for the quantitative measurement of folic acid. The unknown folic acid samples or folic acid standards are first added to a Folic Acid Conjugate preabsorbed microplate. After a brief incubation, an Anti-Folic Acid antibody is added, followed by an HRP conjugated secondary antibody. The folic acid content in unknown samples is determined by comparison with a predetermined folic acid standard curve.

Related Products

1. MET-5054: L-Amino Acid Assay Kit
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
4. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit

5. STA-674: Glutamate Assay Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-Folic Acid Antibody (500X) (Part No. 50681C): One 10 µL vial.
3. Secondary Antibody, HRP Conjugate (Part No. 231009): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. Folic Acid Standard (Part No. 50682C): One 100 µL amber vial of 10 µg/mL Folic Acid in water.

Box 2 (shipped on blue ice packs)

1. 100X Folic Acid Conjugate (Part No. 50683C): One 100 µL amber vial.

Materials Not Supplied

1. Folic acid samples such as serum, plasma, or folic acid extracted from cells or tissues
2. Tissue Homogenizer
3. 1X PBS
4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Multichannel micropipette reservoir
7. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store 100X Folic Acid Conjugate at -20ºC and avoid multiple freeze/thaw cycles. Store all other components at 4ºC. The 100X Folic Acid Conjugate and Folic Acid Standard are light sensitive and must be stored accordingly.

Preparation of Reagents

- Folic Acid Conjugate Coated Plate: Dilute the proper amount of 100X Folic Acid Conjugate 1:100 into 1X PBS. Add 100 µL of the diluted 1X Folic Acid Conjugate to each well and incubate at 37ºC for two hours or overnight at 4ºC. Remove the coating solution and wash twice with 200 µL of 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4ºC and remove the Assay Diluent immediately before use.

Note: The Folic Acid Conjugate-coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Folic Acid Antibody and Secondary Antibody: Immediately before use dilute the Anti-Folic Acid Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Use the provided stock Folic Acid Standard 10 µg/mL solution to prepare a series of the remaining standards according to Table 1 below.

Standard Tubes	10 µg/mL Folic Acid Standard (µL)	Assay Diluent (µL)	Folic Acid (ng/mL)	Folic Acid (nM)
1	10	990	100	227
2	100 of Tube #1	300	25	56.75
3	100 of Tube #2	300	6.25	14.19
4	100 of Tube #3	300	1.56	3.55
5	100 of Tube #4	300	0.391	0.887
6	100 of Tube #5	300	0.098	0.222
7	100 of Tube #6	300	0.024	0.055
8	0	300	0	0

Table 1. Preparation of Folic Acid Standards.

Preparation of Samples

- Serum: Avoid hemolyzed and lipemic blood samples. Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent or PBS containing 0.1% BSA as necessary.
- Plasma: Avoid hemolyzed and lipemic blood samples. Collect blood with heparin or citrate and centrifuge at 2000 x g and 4ºC for 10 minutes. Remove the plasma layer and store on ice. Avoid disturbing the white buffy layer. Aliquot samples for testing and store at -80ºC. Perform dilutions in Assay Diluent or PBS containing 0.1% BSA as necessary.

  Note: This assay is not compatible with rabbit serum or plasma due to high levels of rabbit IgG that will cross react with the secondary antibody.
- Cells or tissues: Homogenize 50-200 mg of the cell pellet or tissue in 0.5-2 mL of ice-cold PBS using a mortar and pestle or by dounce homogenization. Incubate the homogenate at 4°C for 20 minutes. Transfer the homogenate to a centrifuge tube and centrifuge at 12000 x g for 20 minutes. Recover the supernatant and transfer to a fresh tube. Store resuspended sample at -20°C or colder until ready to test by ELISA. Perform dilutions in Assay Diluent or PBS containing 0.1% BSA as necessary.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each folic acid sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or Folic Acid standards to the wells of the Folic Acid Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted Anti-Folic Acid antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Enzyme Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Folic Acid ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

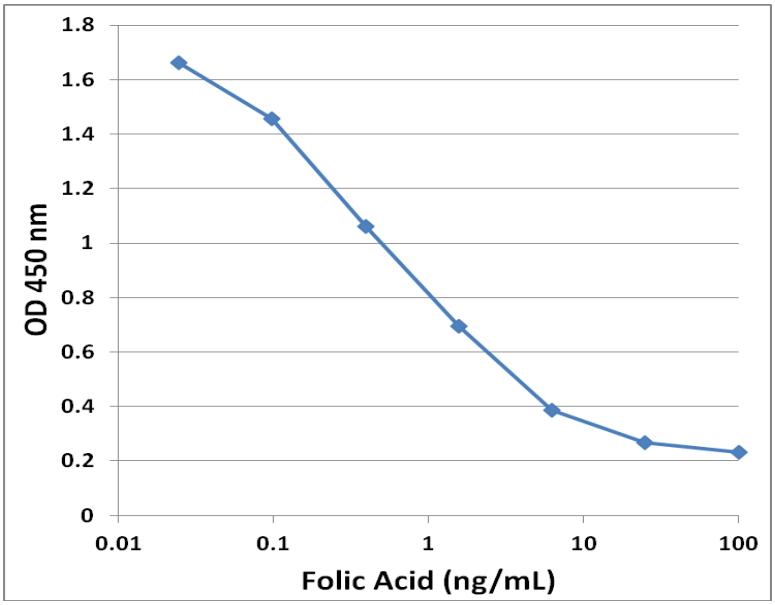

Figure 1: Folic Acid ELISA Standard Curve.

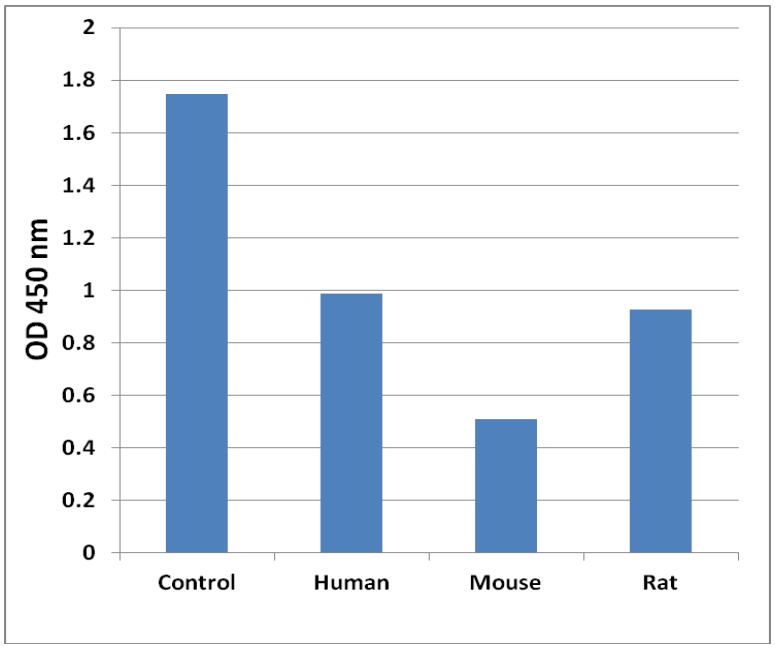

Figure 2: Folic Acid Levels in Human, Mouse or Rat Serum compared to Negative Control (Assay Diluent). Serum samples were diluted 1:5 in Assay Diluent and tested according to the Assay Protocol.

References

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Recent Product Citations

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5. Zhu, J. et al. (2018). Effect of maternal folic acid supplementation on prostatitis risk in the rat offspring. *Int Urol Nephrol*. **50**(11):1963-1973. doi: 10.1007/s11255-018-1969-8.

Warranty

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