Folic Acid / Vitamin B9 ELISA Kit

ARG83469 Folic Acid / Vitamin B9 ELISA Kit is an Enzyme Immunoassay kit for the quantification of All species Folic Acid / Vitamin B9 in Serum, Plasma, Tissue Homogenates, Cell Lysates, Cell Culture Supernates or Other Biological Fluids

Catalog number: ARG83469

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Vitamin B9 is required by the body for the synthesis of purines, pyrimidines, and methionine before incorporation into DNA or protein. Vitamin B9 is particularly important during phases of rapid cell division, such as infancy, pregnancy, and erythropoiesis, and plays a protective factor in the development of cancer. As humans are unable to synthesize Vitamin B9 endogenously, diet and supplementation is necessary to prevent deficiencies.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative competitive enzyme immunoassay technique. Folic Acid / Vitamin B9 monoclonal antibody is bind on the surface of a microtiter plate. Folic Acid / Vitamin B9 containing samples or standards, Folic Acid / Vitamin B9-Peroxidase and an antibody directed against Folic Acid / Vitamin B9 are given into the wells of the microtiter plate. Immobilized and free Folic Acid / Vitamin B9 compete for the antibody binding sites. After incubation, the wells are washed with diluted washing solution to remove unbound material. Then a substrate solution is added and incubated, and measured at 450 nm. The concentration of Quinolones is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C
Standard (Lyophilized)	2 X 250 ng	4°C
Standard/Sample Diluent Buffer	30 ml	4°C
100X Biotin Conjugate	60 µL	4°C
Biotin Conjugate Diluent Buffer	12 ml	4°C
100X HRP-Streptavidin Concentrate	120 µL	4°C
HRP-Streptavidin Diluent Buffer	30 ml	4°C
20X Wash buffer	30 ml	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.
- Briefly spin down the 100X Biotin Conjugate and 100X HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Saliva- Collect saliva using a collection device (e.g. Salivette), centrifuge 10,000 x g for 2 min at 4°C. Collect saliva and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles. The collection device should not have protein binding or filtering features.

Urine - Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Biotin conjugate**: 20 minutes before use, dilute 100X Biotin conjugate with Biotin Conjugate Diluent Buffer to yield **1X Biotin conjugate**.
- **1X HRP-Streptavidin Solution**: 20 minutes before use, dilute 100X HRP-Streptavidin concentrate solution with Streptavidin-HRP Diluent Buffer to yield **1X HRP-Streptavidin** Solution buffer. Keep diluted HRP-Streptavidin Solution in dark before use.
- **Sample**: If the initial assay found samples contain Folic Acid / Vitamin B9 higher than the highest standard, the samples can be diluted with Standard/Sample diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
- **Standards:** Reconstitute the standard with **1 ml Standard/Sample Diluent Buffer** to yield a stock concentration of **250 ng/ml**. Keep the buffer in the vail for at least 15 min at RT to make sure the Standard is dissolved completely before making serial dilutions. The Standard/Sample diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **250 ng/ml**, **125 ng/ml**, **62.5 ng/ml**, **31.3 ng/ml**, **15.6 ng/ml**, **7.8 ng/ml**, **3.9 ng/ml**. DO NOT reuse the reconstituted standard.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-28 °C) before use. Standards, samples and controls should be assayed in duplicates.

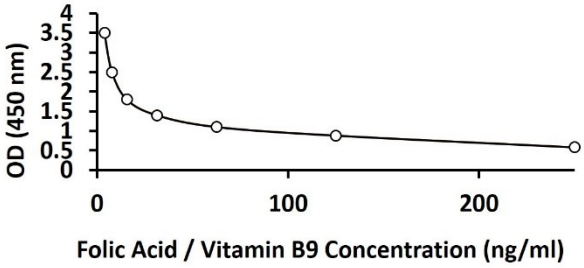
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of **standards** or prepared **samples** into wells.
3. Add **50 µl** of **1 X Biotin conjugate** into wells.
4. Incubate for **60 minutes** at 37°C.
5. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (200µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of **1X HRP-Streptavidin Solution** to each well.
7. Incubate for **45 minutes** at **37°C**.
8. Wash as step 5, but for total 5 washes.
9. Add **100 µl** of **TMB Reagent** to each well.
10. Incubate for **10-20 minutes** at **37°C**.
11. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow.
12. Read the OD with a microplate reader at 450 nm immediately (optional: Read the OD at 600-690nm as reference). It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If these quality control criteria are not met the assay run is invalid and should be repeated.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The standard range of Quinolones ranged from 3.9 -250 ng/ml. The mean Limit of detection was 1.23 ng/ml.