ab285228 – Folic Acid ELISA Kit

For quantitative measurement of folic acid in serum, urine and tissues (e.g. pork, liver, chicken, fish and shrimp)
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285228

Storage and Stability

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 1 month at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Microplate	8 x 12 wells	-20°C
Folic Acid Standard	2 vials	-20°C
HRP-conjugate Stock	25 µL	-20°C
Antibody	7 mL	-20°C
TMB substrate	10 mL	-20°C
Stop Solution	10 mL	-20°C
Sample Diluent	20 mL	-20°C
Wash Buffer (10X)	50 mL	-20°C
Serum Solution	1 mL	-20°C
Standard Buffer	40 mL	-20°C
Conjugate Buffer	7.5 mL	-20°C
Plate Sealers	4 units	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Precision pipettes with disposable tips
- Clean Eppendorf tubes for preparing standards or sample dilutions

Reagent Preparation

- Bring all reagents to room temperature before use.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

TMB Substrate, Stop Solution and Sample Diluent: Ready to be used. After use, store them at 4°C.

Serum Solution: Ready to use. Bring bottle to room temperature before use. Store at -20°C

Wash Buffer (10X): Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.

HRP-conjugate Working Solution: Spin briefly before opening the tube. Pipet 20 µl of HRP-conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the conjugate solution bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.

Standard Preparation

Folic Acid Standard: Add 1 ml of Standard Buffer into a Folic Acid Standard to prepare 25 µg/ml stock. Dilute the stock by 100 folds (e.g. 10 µl in 990 µl of buffer) to prepare the S5 standard (250 ng/ml) in below. Perform 2-fold dilution of S5 for S4 standard (eg. 400 µl in 400 µl of buffer). Perform 5-fold serial dilutions from S5 (e.g. 200 µl in 800 µl of buffer) to prepare S3 to S1 standards sequentially. S0 is the Standard Buffer only. These standards can be stored at -20°C for 2 weeks.

Strandards	S0	S1	S2	S3	S4	S5
Concentrations (ng/mL)	0	2	10	50	125	250

Sample Preparation

- Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
- Avoid multiple freeze-thaw cycles.

Serum:
Add 15 µl of Serum Solution into 285 µl of serum in an eppendorf tube and vortex well.
Incubate the sample at 37°C for 45 min.
Incubate the sample at 85-90°C for 10 min.
Dilute the sample 10 fold with Sample Diluent. For example, mix 100 µl of treated serum with 900 µl of Sample Diluent.
Use 50 µl per well for the assay.
Δ Note: *Dilution factor: 10*

Urine:
Centrifuge 0.5 ml of urine at 10,000 x g for 5 min and recover the supernatant.
Dilute the supernatant 10 fold with Sample Diluent. For example, mix 100 µl of urine with 900 µl of Sample Diluent.
Use 50 µl per well for the assay.
Δ Note: *Dilution factor: 10*

Tissue (pork, liver, chicken, fish and shrimp):
Homogenize 0.1 g of tissue sample with 0.5 ml of Sample Diluent. Vortex for 5 min.
Centrifuge the sample at 10,000 x g, 4°C for 15 min and recover the supernatant.
Dilute the supernatant by 10 fold with Sample Diluent. For example, mix 100 µl of the supernatant with 900 µl of Sample Diluent.
Use 50 µl per well for the assay.
Δ Note: *Dilution factor: 10*

Assay Procedure

- It is recommended that all standards and samples be run at least in duplicate.
- A standard curve should be run for each assay.

1. Prepare all reagents, samples, and standards.
2. Add 50 µl of Standards or Samples per well. Then add 50 µl of conjugate working solution and 50 µl of Antibody to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 4 times: add 250 µl of 1X Wash Buffer and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (This is essential for accurate results.) Repeat this step 3 more times. Remove the last wash by aspiration.
5. Add 100 µl of TMB Substrate to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no folic acid (S0). When its reading is approximately between 1.0 and 1.1 (usually between 15-30 min after adding the TMB Substrate), add 50 µl of Stop Solution and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm for the standards and samples within 10 min.

Calculations

The Standard Curve is done by plotting the OD at 450 nm vs. folic acid concentration. The concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration in the starting sample before dilution.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus