VitaFast® Folic Acid P1001

Total content (natural + added folic acid) in food (see 6.5. manual)

Sample extraction

- weigh exactly 1 g (ml) homogenized sample and 20 mg pancreatin into a 50 ml sterile centrifuge vial
- add 30 ml phosphate buffer (0.05 mol / l; 0.1 % ascorbate; pH 7.2), shake well and fill up to 40 ml with phosphate buffer
- incubate 2 h at 37 °C (98.6 °F) in the dark (shake at times); thereafter heat 30 min at 95 °C (203 °F) in a water bath; chill down quickly to below 30 °C (86 °F)
- transfer 1 ml of the sample extraction in a 1.5 ml sterile reaction vial and centrifuge 5 min (greater than 8,000 x g)



Assay - medium

- remove the desiccant using tweezers
- add 10 ml of sterile water (from test kit) to the medium bottle
- heat 5 min at 95 °C (203 °F), chill down quickly to below 30 °C (86 °F)
- filter through a 0.2 µm filter into a sterile 15 ml centrifuge vial



Sample dilution

- calculate sample dilutions
- fill out microtiter plate manager
- dilute the clear supernatant from the extracted sample with sterile water (from test kit) in 1.5 ml reaction vials

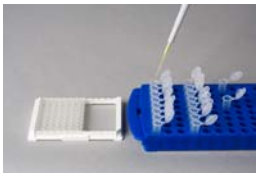
Standard curve

- reconstitute standard with x ml sterile water (from test kit), x = see bottle
- shake and prepare standard curve (take 1.5 ml sterile reaction vials)

standard curve in µg / 100 g (ml)	sterile water in µl		standard concentrate in µl		total volume in µl
blank: 0	900	+	0	=	900
standard 1: 0.16	900	+	100	=	1000
standard 2: 0.32	400	+	100	=	500
standard 3: 0.64	300	+	200	=	500
standard 4: 0.96	200	+	300	=	500
standard 5: 1.28	100	+	400	=	500

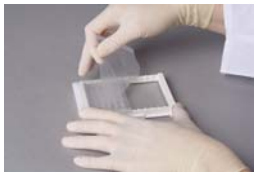

Microtiter plate

- transfer the required strips into the additional holder
- return the unused strips together with the desiccant to the foil bag and seal it well
- pipette assay - medium: **150 µl**
- pipette standard / diluted sample: **150 µl**
  (for each standard / diluted sample solution use new tip)
- cover the strips well with adhesive foil
- incubate **44 - 48 h** at **37 °C (98.6 °F)** (in the dark)


Evaluation

- prepare data file software, put in data (standards, samples, dilutions)
- press down the adhesive foil once more
- place the microtiter plate upside down on a table and dissolve the microorganisms thoroughly by shaking the plate on the surface of the desk
- invert the plate to the regular position and remove the adhesive foil (**hold strips in the frame**)
- disturb the bubbles by means of tip or needle
- read out (610 - 630 nm, alternatively at 540 - 550 nm), use 4 - parameter software (blank has not to be substrated)


Disposals needed

- sterile graduated centrifuge vials, 15 and 50 ml
- sterile reaction vials, 1.5 ml
- sterile tips for micropipettes, 20 - 200 µl and 100 - 1000 µl
- sterile filters polyethersulfon 0.2 µm with syringe

Reagents additionally needed

- phosphate buffer (0.05 mol / l, 0.1 % ascorbate; pH 7.2): solve 7.8 g sodium dihydrogen phosphate dihydrate and 1 g sodium ascorbate in 1 liter redist. or deionized water, adjust pH 7.2
- pig pancreatin (e. g. Sigma P1750); remark: (for samples like grain and yeast products chicken pancreatin is recommended, e. g. Difco 245910)

- read the test insert of VitaFast® Folic Acid carefully
- the extraction procedure above is described for the total content of folic acid in food
- further extraction procedures of added folic acid in liquid samples (6.1), of added folic acid in fruit gums and candies (6.2), of added folic acid in capsules, pills and vitamin mixes (6.3) and of added folic acid in cereals, baby food, bread, flour (6.4) are described in the manual