

AC P Vitamin Folic Assay Broth, Base

For the microbiological assay of vitamins in drugs, foodstuffs, animal feed preparations and other materials.

Certain species of bacteria and some yeasts can only grow in the presence of certain vitamins. If these "test organisms" are transferred to defined culture media which contain all the compounds essential for their growth apart from the vitamin in question, proliferation of the test organisms is totally inhibited or at least drastically reduced. After adding the vitamin the organism can then grow, its growth being dependent on the concentration of the vitamin. The amount of vitamin present can be determined by measuring the turbidity produced as a result of microbial growth or by quantitative assay of a metabolite (e.g. lactic acid). Parallel assays with a pure vitamin preparation of known activity serve as standards.

Typical Composition (g/litre)

D(+)-Glucose, anhydrous 40 g; Casein hydrolysate "Vitamin-free" 12 g; DL-Alanine 400 mg; L-Asparagine 200 mg; L-Cysteinium chloride ; L-Cysteine 200 mg; L-Tryptophane 100 mg; Adenine 20 mg; Guanosin 40 mg; Uracil 20 mg; Xanthine 10 mg; 4-Aminobenzoic acid 200 µg; L(+)-Ascorbic acid ; D(+)-Biotin (Vitamin H) 0.8 µg; Calcium D(+)-pantothenate 400 µg; Folic acid; Nicotin acid 2 mg; Pyridoxol hydrochloride 4 mg; Pyridoxamine hydrochloride ; Riboflavin 2 mg; Thiaminium dichloride 2mg; di-potassium hydrogen phosphate 1 g; Iron(II) sulfate 20 mg; Potassium dihydrogen phosphate 1 g; Magnesium sulfate 400 mg; Manganese(II) sulfate 20 mg; tri-sodium citrate dihydrate 30 g; Sodium acetate, anhydrous 20 g; Sodium chloride 20mg; To be added: Tween® 80 0.4 ml; pH at 25 °C (± 0.1) 6.8; Quantity per litre (preparation) 106 g

Sample preparation

Folic Acid Test

Extraction	Folic acid is extracted from the examination material by water and by adding an equivalent amount of caustic soda solution and heating. Enzymatic pre-treatment is necessary to release bound folic acid. For this, homogenize 1 g of the sample material in 150 ml of 0.05 N phosphate buffer (pH 7.2) and sterilize (15 min at 121 °C). Add 20 mg pancreatin (dried) and incubate for 24 hours at 37 °C. Then the suspension is autoclaved (5 min at 121 °C), cooled and filtered.
Inoculation culture	<i>Enterococcus hirae</i> (ATCC 8043) from the type culture of the test organism is inoculated in Micro-Inoculum Broth and incubated for 24 hours at 37 °C. Then the culture is centrifuged and rinsed several times with physiological saline and adjusted to a microbial count of $2 \cdot 10^8$ bacteria/ml.
Calibration	Suspend 50 mg dried folic acid in 30 ml 0.01 N caustic soda solution. Add 300 ml bidistilled water and adjust the pH to 7.8 (± 0.5) filling up to 500 ml (content: 100 mcg/ml). Before use, this stock solution is diluted to 2 ng/ml to give the reference solution. For calibration, a concentration series of 0.0-0.5-1.0-2.0-4.0-8.0-10.0 ng folic acid per 10 ml is made by pipetting 0.0-0.25-0.5-1.0-2.0-4.0-5.0 ml of reference solution into test tubes and filling up to 5.0 ml with bidistilled water. Test tubes for culture and sterility controls only contain 5 ml of water.
Sample	As with the reference solution, also the sample solution is prepared in a reducing series in test tubes filled up to 5 ml with bidistilled water.
Preparation of test culture medium inoculation	By briefly boiling, dissolve 106 g of dehydrated Vitamin Folic Acid Assay Test Broth together with 0.4 ml Tween® 80 in 1 litre bidistilled water. Check the pH and when required correct it (6.8 at 25 °C). Add 5 ml of culture medium to all test tubes with control, sample or reference solution, close with caps and sterilize by autoclaving (10 min at 115 °C). After cooling inoculate the test tubes (apart from sterile controls) with 1 drop of inoculation culture. Incubate for 16 to 20 hours at 37 °C.
Evaluation	The optical density (OD) of the reference and sample batches is measured photometrically at 546nm against the culture control. A calibration curve is made by applying the turbidity values on the linear ordinate to the corresponding active substance amounts on the logarithmic abscissa. An evaluation is only worthwhile at OD (546 nm, 1 cm) < 0.0150 for the control culture measured against water. The sterile controls must not show any growth.

Vitamin Folic Assay Broth, Base

Micro-Inoculum Broth

Typical Composition (g/litre)

Proteose peptone 5.0; Yeast extract 20.0; D(+)glucose 10.0;
Potassium dihydrogen phosphate 2.0; Tween® 80 0.1

Micro Assay Culture Agar

Preparation

Add 10 g agar-agar to the Micro-Inoculum Broth, autoclave for 15 min at 121 °C.

pH: 6.7 ± 0.1 at 25 °C

Incubation: 24 hours at 35 °C aerobically (both media).

Ordering Information

Product	Merck Cat. No.	Pack size
Vitamin Folic Assay Broth, Base	1.11990.0100	100 g
α-Amylase	1.01329.0001	1 g
0.2 N Sodium hydroxide solution	1.09140.1000	1 l
Acetate buffer solution pH 4.66	1.07827.1000	1 l
Agar-agar purified	1.01614.1000	1 kg
Calcium D(+)pantothenate	1.02316.0010	10 g
Chloroform	1.02445.0250	250 ml
Citric acid monohydrate	1.00244.0500	500 g
D(+)Biotin (Vitamin H)	1.24514.0001	1 g
di-sodium hydrogen phosphate	1.06586.0500	500 g
Folic acid for biochemistry	1.03984.0005	5 g
Hydrochloric acid 0.5 N	1.09058.1000	1 l
Nicotinamide	1.06818.0100	100 g
Nicotinic acid	1.06817.0100	100 g
Pancreatin DAB	1.07133.0500	500 g
Papain, water-soluble	1.07144.0025	25 g
Sodium acetate, anhydrous	1.06268.0250	250 g
Sodium chloride	1.06404.0500	500 g
Sodium disulfite	1.06528.0100	100 g
Sodium hydroxide solution 0.1 N	1.09141.1000	1 l
Sodium hydroxide solution 1 mol/l	1.09137.1000	1 l
Sulfuric acid 1.0 N	1.09072.1000	1 l
Toluene	1.08325.1000	1 l
Tween® 80	8.22187.0500	500 ml
Vitamin B ₁₂ (cyanocobalamin)	1.24592.0100	100 mg

Quality control

Test strains	Inoculation culture	Growth
Enterococcus hirae ATCC 8043	Adjusted on 50 % T (630 nm, 1 cm cuvette, against 0.9 % NaCl)	Calibration curve shows graduated growth between 200 to 2000 pg folic acid