

Vitamin Pantothenic Acid 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 ; L-Asparagine ; L-Cysteinium chloride ; L-Cysteine 400 mg; L-Tryptophane 100 mg; Adenine 20 mg; Guanosin 40 mg; Uracil 20 mg; Xanthine ; 4-Aminobenzoic acid 200 µg; L(+)-Ascorbic acid ; D(+)-Biotin (Vitamin H) 0.8 µg; Calcium D(+)-pantothenate ; Folic acid ; Nicotin acid 1 mg; Pyridoxol hydrochloride 800 µg; Pyridoxamine hydrochloride ; Riboflavin ; Thiaminium dichloride 200 µg; 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 ; Sodium acetate, anhydrous 20 g; Sodium chloride 20 mg; To be added: Tween® 80 0.4 ml; pH at 25 °C (± 0.1) 6.8; Quantity per litre (preparation) 75 g

Sample preparation

Pantothenic Acid Test

Extraction	To determine pantothenic acid and its salts in materials where the general quantity is known (e.g. pharmaceutical preparations such as injection solutions, tablets, dragees etc.), an aqueous extraction or solution is diluted so that the expected turbidity values lie in the middle range of the calibration curve. If the content of free vitamin is totally unknown, it is recommended to carry out a pre-examination with a concentrated extract to the material to determine the general quantity. If the vitamin is present in bound form (in natural material such as vegetable or animal samples), it must be previously released completely by enzymatic hydrolysis.
Enzymatic Hydrolysis	Homogenize 1 g of examination material in 80 ml of acetate buffer solution. Add 40 mg papain and 40 mg amylase (diastase) and a few drops of toluene or chloroform to the suspension. Maintain at 37 °C for about 24 hours, then heat the substrate at 100 °C for 30 minutes. After cooling adjust pH to 6.8 with caustic soda solution, fill up to 100 ml with standard acetate buffer solution and filter.
Inoculation culture	Lactobacillus plantarum (ATCC 8014) from the type culture of the test organism is inoculated in Micro-Inoculum Broth or in the semi-concentrated (37.5 g/l) culture medium with 20 ng/ml of added calcium pantothenate and incubated for 20 hours at 37 °C. Then the culture is centrifuged and rinsed several times with physiological saline and adjusted to a microbial count of $3 \cdot 10^8$ bacteria/ml.
Calibration	Suspend 50 mg calcium-D-pantothenate in 50 ml of bidistilled water (content: 1 mg/ml). This stock solution is diluted before use by the power of ten down to 20 ng/ml as the reference solution. For calibration a concentration series of 0-10-20-40-60-80-100 ng calcium-D-pantothenate per 10 ml is made by pipetting 0.0-0.5-1.0-2.0-3.0-4.0-5.0 ml of the 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 deducing series in test tubes filled up to 5 ml with bidistilled water.
Preparation of test culture medium inoculation	By briefly boiling, dissolve 75 g of dehydrated Vitamin Pantothenic Acid Assay Broth together with 0.4 ml Tween® 80 in 1 litre bidistilled water. Check the pH and when required correct (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 24 hours at 37 °C.
Evaluation	The optical density (OD) of the reference and sample batches is measured photometrically at 546 nm 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) < 0.150 for the control culture measured against water. The sterile controls must not show any growth.

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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

Preparation

Suspend 37.1 g in 1 litre of demin. water, autoclave 15 min at 121 °C.

pH: 6.7 ± 0.2 at 25 °C

The medium is clear and brown.

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 Pantothenic Acid Assay Broth, Base	1.11993.0100	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
Lactobacillus plantarum ATCC 8014	Adjusted on 50 % T (630 nm, 1 cm cuvette, against 0.9 % NaCl)	Calibration curve shows graduated growth between 10 to 60 ng Ca-pantothenate