Vitamin Biotin Assay Broth

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 200 mg; L-Tryptophane 100 mg; Adenine 20 mg; Guanosin 40 mg; Uracil 20 mg; Xanthine 10 mg; 4-Amino-benzoic acid 200 µg; L(+)Ascorbic acid ; D(+)Biotin (Vitamin H); Calcium D(+)pantothenate 2 mg; Folic acid ; Nicotin acid 2mg; Pyridoxol hydrochloride 4 mg; Pyridoxamine hydrochloride ; Riboflavin 2 mg; Thiaminium dichloride 2 mg; 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 ; pH at 25 °C (± 0.1) 6.8; Quantity per litre (preparation) 75 g;

Sample preparation

D-Biotin (Vitamin H) Test

Extraction	To determine the D-biotin content of examination material where the general quantity is known (e.g. pharmaceutical products), the sample under examination is homogenized in water with heating. Pre-examination to establish the general quantity is recommended, if the biotin content is completely unknown. For this, if possible, a concentrated extract is prepared and examined in a dilution series reducing at at the power of 10. If the biotin is bound (e.g. in natural vegetable products) it will be released by acid hydrolysis.
Acid hydrolysis	Homogenize 1 g of examination material in 50 ml 1 N sulfuric acid and then autoclave for 2 hours at 121 °C. After cooling, adjust pH to 4.5, centrifuge and pipette off the supernatant to remove undissolved components. Dilute with distilled water to an optimum concentration for the test. To release biotin from animal material, the autoclavation can be redued to 1 hour at 121 °C, if a stronger acid such as 6 N sulfuric acid is used.
Inoculation culture	Lactobacillus plantarum (ATCC 8014) 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 three times with physiological saline and adjusted to a microbial count of $3 \cdot 10^8$ bacteria/ml.
Calibration	Suspend 100 mg D-biotin, warming in a steam bath, in 1 litre of bidistilled water (content: 100mcg/ml). Before use, this stock solution is diluted to 1 ng/ml to give the reference solution. For calibration a concentration series of 0.0-0.2-0.4-0.8-1.0-1.5-2.0-2.5-3.0 ng D-biotin per 10ml is made by pipetting 0.0-0.2-0.4-0.8-1.0-1.5-2.0-2.5-3.0 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 reducing 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 Biotin Assay Broth in 1 litre bidistilled water. Check the pH and if required correct (6.8 at 25 °C). Add 5 ml of the 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 c) < 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

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 Biotin Assay Broth	1.11989.0100	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 30 % T (630 nm, 1cm cuvette, against 0.9 % NaCl)	Calibration curve shows gradiated growth between 0.2 to 3 ng biotin