Vitamin B_{12} (Lactobacillus) 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" 15 g;DL-Alanine ; L-Asparagine 200 mg; L-Cysteinium chloride 200 mg; L-Cysteine 400 mg; L-Tryptophane 200 mg; Adenine 20mg; Guanosin 40 mg; Uracil 20 mg; Xanthine 20 mg; 4-Aminobenzoic acid 2 mg; L(+)Ascorbic acid 4 g; D(+)Biotin (Vitamin H) 10 µg; Calcium D(+)pantothenate 1 mg; Folic acid 200 µg; Nicotin acid 2 mg; Pyridoxol hydrochloride 4 mg; Pyridoxamine hydrochloride 800 µg; Riboflavin 1 mg; Thiaminium dichloride 1 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 2 ml; pH at 25°C (± 0.1) 6.0; Quantity per litre (preparation) 83 g

Sample preparation

Vitamin B_{12} Test with Lactobacillus delbrueckii var. lactis

Extraction	If Vitamin B_{12} is freely available in the examination material (e.g. powders, tablets) a simple water extraction is quite adequate. Should the material also contain bound Vit. B_{12}, decomposition either with buffer solution or enzymatic hydrolysis is necessary.
Buffer solution	Homogenize 1 g of examination material in 50 ml of buffer solution (composition: 1.29 g di-sodium hydrogen phosphate, 1.1 g citric acid and 1.0 g sodium disulfite dissolved in 100 ml of demin. water), autoclave for 10 minutes at 121 °C. Adjust pH to 6.0 after cooling, fill up to 100ml with sterile distilled water, filter or centrifuge.
Enzymatic Hydrolysis	Homogenize 1 g of examination material in 80 ml of acetate buffer solution. Add papain, amylase (diastase) and a few drops of toluene or chloroform to the suspension. Maintain at 37°C for about 24 hours, then heat at 100 °C for 30 minutes. After cooling adjust pH to 6.6 with caustic soda solution and fill up to 100 ml with standard acetate buffer solution. The suspension is either filtered or centrifuged. A pre-examination is recommended, if the content of Vit. B_{12} is completely unknown. For this, if possible, a concentrated extract is prepared and examined in a dilution series reducing at the power of 10.
Inoculation Culture	Lactobacillus delbrueckii var. lactis (ATCC 7830) from the type culture of the test organisms is inoculated in Micro-Inoculum-Broth and incubated for 20 hours at 37 °C. Then the culture is centrifuged and rinsed three times with physiological saline and adjusted to a microbial count of 10^8 bacteria/ml.
Calibration	Suspend 100 mg of dried cyanocobalamin (Vitamin B12) in 1 litre of bidistilled water (content: 100 mcg/ml). Before use, this stock solution is diluted to 100 pg/ml to give the reference solution. For calibration a concentration series of 0-25-50-75-100-125-150-200-500 pg cyanocobalamin per 10 ml is made by pipetting 0.0-0.25-0.50-0.75-1.0-1.25-1.5-2.0-5.0 ml of reference solution into test tubes and filling up to 5.0 ml with bististilled 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 83 g of dehydrated Vitamin B_{12} (Lactobacillus) Assay Broth together with 2 ml Tween® 80 in 1 litre bidistilled water. Check the pH and correct if necessary (6.0 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 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) < 0.150 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 B_{12} (Lactobacillus) Assay Broth, Base	1.11988.0100	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 cultures	Growth
Lactobacillus delbrueckii var. lactis ATCC 7830	Adjusted on 50 % T (630 nm, 1 cm cuvette, against 0.9 % NaCl)	Calibration curve shows gradiated growth between 25 to 300 pg cyanocobalamin