82897 Vitamin B_{12} Assay Medium

For the microbiological assay of vitamins B_{12} in drugs, foodstuffs, animal feed preparations and other materials.

Composition:

Ingredients	Grams/Litre
D(+)-Glucose anhydrous	40.0
Casein hydrolysate "Vitamin-free"	15.0
L-Asparagine	0.2
L-Cystinium chloride	0.2
L-Cystine	0.4
DL-Tryptophan	0.4
Adenine	0.02
Guanine	0.02
Uracil	0.02
Xanthine	0.02
4-Aminobenzoic acid	0.002
L(+)-Ascorbic acid	4.0
D(+)-Biotin (Vitamin H)	0.00001
Calcium D(+)-pantothenate	0.001
Folic acid	0.0002
Nicotinic acid	0.002
Pyridoxal hydrochloride	0.004
Pyridoxamine hydrochloride	0.0008
Riboflavin	0.001
Thiaminium dichloride	0.001
Potassium phosphate dibasic	1.0
Iron(II)sulfate	0.02
Potassium phosphate monobasic	1.0
Magnesium sulfate	0.4
Manganese(II) sulfate	0.02
Sodium acetate anhydrous	20.0
Sodium chloride	0.02

Final pH 5.6 +/- 0.2 at 25°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:

Sample preparation:

In case all the vitamin B_{12} is present in a free form the examination material (e.g. powders or levigated tablets) can be simply extracted with water. Should the sample contain bonded vitamin B_{12}, decomposition with buffer solution or enzymatic hydrolysis is necessary.

Decomposition with buffer solution:
Buffer: Dissolve 1.29 g disodium hydrogen phosphate, 1.1 g citric acid and 1.0 g sodium metabisulfite in 100 ml of distilled water.
Homogenize 1 g of sample in 50 ml buffer and autoclave for 10 minutes at 121°C. Let cool down and adjust the pH to 6.0, fill up to 100 ml with sterile distilled water. Filter or centrifuge to get a solution without particles.

Decomposition with enzymatic hydrolysis
Homogenize 1 g sample in 80 ml of acetate buffer. Papain (76222), amylase (10080) and a few drops of chloroform or toluene are added to the homogeneous suspension. The two enzymes can be replaced by a corresponding diastase. Incubate for about 24 hours at 37°C, then heat for 30 minutes at 100°C. Let cool down and adjust the pH to 6.6 with a sodium hydroxide solution and fill up to 100 ml with acetate buffer (72749; pH 4.65). The suspension is filtered or centrifuged to separate particles. It is recommended to perform a preliminary test, if the content of vitamin B_{12} is completely unknown. For this preliminary test, a concentrated extract is prepared and examined in different dilutions, a dilution factor of 10 is recommended.

Preparatory culture of test organism
Lactobacillus delbrueckii subsp. *lactis* (ATCC 7830) is used as a test organism. Inoculate the *Lactobacillus* in Micro-Inoculum-Broth (composition: Proteose peptone 5 g/l, yeast extract 20 g/l, D(+)-glucose 10 g/l, potassium dihydrogen phosphate 2 g/l, Tween 80 0.1 g/l) and incubate for 20 hours at 37°C. Then centifuge the culture and wash three times with physiological saline and adjust to a microbial count of 10^8 bacteria/ml.

Calibration
Dissolve 100 mg of vitamin B_{12} (Sigma V2876) in 1 litre of distilled water (100 µg/ml). This stock solution is diluted to 100 pg/ml to give the reference solution.

Prepare the following calibration concentrations:

vitamin B_{12} final test concentration [pg/ml]	0	2.5	5	7.5	10	12.5	15	20	50
Reference solution [ml]	0	0.25	0.5	0.75	1.0	1.25	1.5	2.0	5.0
distilled water [ml]	5	4.75	4.5	4.25	4.0	3.75	3.5	3.0	0.0

End volume: 5 ml

Preparation of sample and controls
Culture and sterility controls only contain 5 ml of water. The samples are also prepared as dilution series and filled up to 5 ml with distilled water.

Test
Dissolve 83 g of dehydrated Vitamin B_{12} Assay Medium together with 2 ml Tween® 80 in 1 litre distilled water. Heat gently to a boil to dissolve the medium completely. Check the pH and correct if necessary to 6.0 (at 25°C). Add 5 ml of culture medium to all tubes with control, sample or calibration solution and close them with caps. Sterilisation for 10 min at 115°C. Let cool down and inoculate all test tubes, excluding the sterile controls, with 1 drop of preparatory culture. Incubate for 24 hours at 37°C.

Examination, evaluation
The calibration standards and samples are measured photometrically at 546 nm against the culture control. A calibration curve is recorded with the optical density (OD) values on the linear ordinate against the vitamin B_{12} concentration on the logarithmic abscissa. To get reproducible results the control culture measured against water should have an $OD_{546\ nm,\ 1\ cm}$ below 0.150. There must be no growth by the sterile controls.

Principle and Interpretation:
The growth of *Lactobacillus delbrueckii* var. *lactis* is limited by the concentration of vitamin B_{12} in this defined medium. Due to the limitation the vitamin B_{12} concentration can be calculated with a calibration curve.

References:

1. AACC, Approved methods of the American Association of Cereal Chemists. 8th edition. American (1994.)
2. U.S. Pharmacopeia 21st rev., p 1183, (1985)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.