B3801 B_{12} Assay Medium (Vitamin B_{12} Assay Medium)

Vitamin B_{12} Assay Medium is recommended for the microbiological assay of Vitamin B_{12} by using *Lactobacillus leichmannii* ATCC 7830 (1) as the test organism.

Composition:

Ingredients	Grams/Litre
Casein Acid Hydrolysate	15.0
Dextrose	40.0
Asparagine	0.2
Sodium Acetate	20.0
Ascorbic Acid	4.0
L-Cystine	0.4
DL-Tryptophan	0.4
Adenine Sulfate	0.02
Guanine Hydrochloride	0.02
Uracil	0.02
Xanthine	0.02
Riboflavin	0.001
Thiamine Hydrochloride	0.001
Biotin	0.00001
Niacin	0.002
p-Amino Benzoic Acid	0.002
Calcium Pantothenate	0.001
Pyridoxine Hydrochloride	0.004
Pyridoxal Hydrochloride	0.004
Pyridoxamine Hydrochloride	0.0008
Folic Acid	0.0002
Monopotassium Phosphate	1.0
Dipotassium Phosphate	1.0
Magnesium Sulfate	0.4
Sodium Chloride	0.02
Ferrous Sulfate	0.02
Manganese Sulfate	0.02
Polysorbate 80	2.0
Final pH 6.1 +/- 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 4°C.

Appearance: Yellow colored powder having a tendency to form soft lumps, which can be easily broken down to powder form.

Color and Clarity: Light amber colored, clear solution which may have a slight precipitate.

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 (S7899; pH 5.2). 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 (0.85% NaCl) and adjust to a microbial count of 10^8 bacteria/ml.

Calibration
Dissolve 100 mg of vitamin B_{12} (Cat. No. 95190) 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	25	50	75	100	125	150
Reference solution [ml]	0	0.25	0.5	0.75	1.0	1.25	1.5
distilled water [ml]	5	4.75	4.5	4.25	4.0	3.75	3.5

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
Suspend 8.5 g of Vitamin B12 Assay Medium in 100 ml of distilled water. Boil to dissolve the medium completely. Mix well to distribute the slight precipitate evenly. Dispense 5 ml of medium into tubes containing increasing concentrations of cyanocobalamin (Cat. No. 95190) standard or the unknown. The total volume of 10 ml per tube is adjusted by adding distilled water. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. Cool the medium. 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 620 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 $OD_{620\ nm,\ 1\ cm}$ below 0.150. There must be no growth by the sterile controls.

Principle and Interpretation:
Vitamin B_{12} Assay Medium is a Vitamin B_{12} free medium containing all other vitamins and nutrients essential for the growth of *Lactobacillus leichmannii* ATCC 7830. Cyanocobalamin (Vitamin B_{12}, Cat. No. 95190) is added in increasing concentrations giving a growth response that can be turbidimetrically or acidimetrically measured.

Cultural characteristics after 18-24 hours at 35-37°C.

Organisms (ATCC)	Growth	Cyanocobalamin
Lactobacillus leichmannii (7830)	+ to +++ (depends on the Cyanocobalamin content)	Standard concentrations: 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15 ng Sample: Shoud be in the same concentration range

Concentrations are determined by absorbance at 620 nm

References:

1. American Type Culture Collection, Manassas, Va..
2. The United States Pharmacopeia, (1990). XX United States Pharmacopeial Convention, Inc. Rockville, Maryland.
3. Official Methods of Analysis, (1995). AOAC International, Vol. 1.

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.