Vitamin B_{12} Assay Medium

Intended Use

Vitamin B_{12} Assay Medium is used for determining vitamin B_{12} concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Vitamin B_{12} Assay Medium is prepared according to the formula described by Capp, Hobbs and Fox.[1] This medium is used in the microbiological assay of vitamin B_{12} using *Lactobacillus delbrueckii* subsp. *lactis* (*Lactobacillus leichmannii*) ATCC™ 4797 or 7830.

Principles of the Procedure

Vitamin B_{12} Assay Medium is a vitamin B_{12}-free medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830. To obtain a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations providing a growth response that can be measured titrimetrically or turbidimetrically.

User Quality Control

Identity Specifications

Difco™ Vitamin B_{12} Assay Medium

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	3.8% solution (single-strength), soluble in purified water upon boiling 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Appearance:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Difco™ Vitamin B_{12} Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 4797 when prepared single strength and supplemented with cyanocobalamin (vitamin B_{12}). The medium should produce a standard curve using a cyanocobalamin reference standard at 0.0 to 0.25 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Formula

Difco™ Vitamin B_{12} Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.2	g
DL-Tryptophan	0.2	g
Adenine	20.0	mg
Guanine	20.0	mg
Uracil	20.0	mg
Xanthine	1.0	mg
Thiamine Hydrochloride	2.0	mg
Riboflavin	2.0	mg
Niacin	2.0	mg
Calcium Pantothenate	200.0	µg
Pyridoxine Hydrochloride	4.0	mg
p-Aminobenzoic Acid	200.0	µg
Biotin	10.0	µg
Folic Acid	100.0	µg
Polysorbate 80	2.0	g
Dipotassium Phosphate	1.0	g
Monopotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 7.6 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 mL.
6. Autoclave at 121°C for 5 minutes.

Procedure

Stock cultures of the test organism, *L. delbrueckii* subsp. *lactis* ATCC™ 4797 or 7830, are prepared by stab inoculation of Lactobacilli Agar AOAC or B_{12} Culture Agar. Following incubation at 37°C for 24-48 hours, the tubes are stored in the refrigerator. Transfers are made at 2 week intervals.

Inoculum for the assay is prepared by subculturing a stock of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830 into a tube containing 10 mL of Lactobacilli Broth AOAC or B_{12} Inoculum Broth. After incubation at 35-37°C for 18-24 hours, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed by resuspending in 10 mL of sterile 0.85% saline solution and centrifuging. The washing is repeated for a total of 3 times. Finally the cells are resuspended in 10 mL of sterile 0.85% saline. The cell suspension is then diluted 1:100 with sterile 0.85% saline. One drop is used to inoculate each assay tube.

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation that influence the standard curve readings cannot always be duplicated.

The concentrations required for the preparation of the standard curve are obtained by adding sufficient 25% ethanol to an accurately weighed amount of USP Cyanocobalamin Reference Standard (resulting in a solution containing 1.0 µg of cyanocobalamin per mL). This stock solution is stored in the refrigerator and should be used within 60 days. In the preparation of the standard curve, further dilutions of this stock solution (1 µg/mL) are made as follows:

A. Add 1 mL stock solution to 99 mL purified water (1 mL = 10 ng).
B. Add 1 mL of the solution from step A to 199 mL purified water (1 mL = 0.05 ng).

An acceptable standard curve can be obtained by using the USP Cyanocobalamin Reference Standard at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 ng per assay tube. This is accomplished by adding 0, 0.5, 1, 2, 3, 4 and 5 mL of the 0.05 ng/mL solution per assay tube and sufficient purified water to make 10 mL volume per tube.

A standard concentration is used which, after incubation, gives a transmittance value at the 5 mL level of not less than that which corresponds to a dry cell weight of 1.25 mg (see *USP*[2] for method of calibration of a spectrophotometer and determination of dry cell weight). For the titrimetric method, a standard concentration should be used which, after incubation, will give a titration at the 5 mL level of 8-12 mL 0.1N sodium hydroxide.

Inoculate and incubate at 35-37°C for 18-24 hours. For turbidimetric determinations, place tubes in a refrigerator at 2-8°C for 15-20 minutes to stop growth. The growth can be measured by a nephelometric method. Titrimetric determinations of growth are made after incubation at 37°C for 72 hours. The curve is then constructed from the values obtained.

Expected Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average. Use the results only if two-thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. Capps, Hobbs and Fox. 1949. J. Biol. Chem. *178*:517.
2. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Vitamin B_{12} Assay Medium

Cat. No. 236010 Dehydrated – 100 g*

**Store at 2-8°C.*