Dehydrated Culture Media
Bases / Media Supplements

Technical Information

Vitamin B12 Assay Medium

Product Code: DM 1036

Application: Vitamin B12 Assay Medium is recommended for microbiological assay of Vitamin B12 by using *Lactobacillus leichmannii* ATCC 7830 as the test organism.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate, vitamin free	15.000
Dextrose	40.000
Asparagine	0.200
Sodium acetate	20.000
Ascorbic acid	4.000
L-Cystine	0.400
DL-Tryptophan	0.400
Adenine sulphate	0.020
Uracil	0.020
Xanthine (Sodium)	0.020
Riboflavin (Vitamin B2)	0.001
Thiamine hydrochloride	0.001
Biotin	0.00001
Niacin	0.002
p-Amino benzoic acid (PABA)	0.002
Calcium pantothenate	0.001
Pyridoxine hydrochloride	0.004
Pyridoxal hydrochloride	0.004
Pyridoxamine hydrochloride	0.0008
Folic acid	0.0002
Monopotassium phosphate	1.000
Dipotassium phosphate	1.000
Magnesium sulphate	0.400
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.020
Polysorbate 80	2.000
Guanine hydrochloride	0.020
Final pH (at 25°C)	6.1±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Lactobacillus species grow poorly on non-selective culture media and require special nutrients for their growth. Vitamin assay media are prepared for microbiological assay of vitamins. Three types of media used for the microbiological assay of vitamins can be used as maintenance media for preserving the stock culture, the inoculums media for preparation of the inoculums and the assay media for quantitation of the vitamin under test. Vitamin B12 Assay Medium is a Vitamin B12 free medium containing all other vitamins and nutrients essential for the growth of Lactobacillus leichmannii ATCC 7830. It was first described by Capp et al [(1)] and is recommended by USP [(2)] and AOAC [(3),] using Lactobacillus leichmannii ATCC 7830. As the test organism. Inoculum for the assay is prepared by subculturing from a stock culture previously made by stab inoculation. Freshly subcultured organisms incubated at 37°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for the assay. The growth response obtained is measured turbidometrically or acidimetrically A standard curve is plotted with absorbance as a function of the vitamin B12 concentration [(2, 3)]. The concentration of vitamin B12 in the test sample is calculated based on the interpretation of the standard curve. Extreme care should be taken to avoid contamination of media or glassware used for the assay. Detergent-free clean glassware should be used. Even small amount of contamination by foreign material may lead to erroneous results. The test organism used for inoculating must be cultured and maintained on media recommended for this purpose.

Methodology

Suspend 8.45 grams of powder media in 100 ml distilled water. Shake well and heat if necessary to dissolve the medium completely. Mix well to distribute the slight precipitate evenly. For the assay, dispense 5 ml medium to each assay tube (containing increasing amounts of standard or the unknown). Total volume of 10 ml per tube is adjusted by addition of distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. Cool the medium immediately. Generally satisfactory results are obtained with Vitamin B12 (Cyanocobalamin) at levels 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 ng per assay tube (10 ml).

Quality Control

Physical Appearance
Cream to yellow homogeneous having a tendency to form soft lumps which can be easily broken down to powder form.

Colour and Clarity of prepared medium
Light amber coloured clear solution that may contain a slight precipitate

Reaction
Reaction of 8.5% w/v aqueous solution at 25°C. pH : 6.1±0.2

pH Range
5.90-6.30

Growth
Gradual increase in growth with increasing USP Cyanocobalamin reference standard levels of 0.0, 0.025, 0.050, 0.075, 0.1, 0.125, 0.150 and 0.2 ng per assay tube is recorded as equivalent increase in absorbance at 620nm.

Cultural Response/Characteristics
DM 1036: Microbiological assay of Vitamin B12 is carried out using Lactobacillus leichmannii ATCC 7830 after an incubation at 35-37°C for 18-24 hours.

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.

Further Reading

1. Capps B. E., Hobbs M. H. H. and Fox S. H., 1949, J. Biol. Chem., 178:517.
2. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopeial Convention, Rockville, MD.
3. H. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conforms solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate.
- **Central Drug House Pvt. Ltd.** reserves the right to make changes to specifications and information related to the products at any time.
- Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing of diagnostic reagents extra.
- Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.
- Do not use the products if it fails to meet specificatons for identity and performens parameters.