



B12 Assay Medium (Using *L. leichmannii*)

M036

Intended Use:

(Vitamin B12 Assay Medium) for microbiological assay of vitamin B12 using *Lactobacillus leichmannii* ATCC 7830 as the test organism.

Composition**

Ingredients	Gms / Litre
Acicase™, vitamin free	15.000
Dextrose (Glucose)	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

Equivalent to Casein acid hydrolysate, vitamin free

Directions

Suspend 8.45 grams in 100 ml purified / distilled water. 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).

Principle And Interpretation

Lactobacillus species grow poorly on non-selective culture media and require special nutrients for their growth. Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media used for the microbiological assay of vitamins are the maintenance media used for carrying the stock culture, the inoculum media for preparation of the inoculum 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 (5) and AOAC (2), using *Lactobacillus leichmannii* ATCC 7830 as the test organism.

Standard curve is constructed with known dilutions of vitamin B12 standards (2,5).

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 turbidometrically or acidimetrically measured.

A standard curve is plotted with absorbance as a function of the vitamin B12 concentration. 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

Type of specimen

Pure isolates.

Specimen Collection and Handling

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 turbidometrically or acidimetrically measured.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

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

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

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

Store dehydrated and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Capps B. E., Hobbs M. H. H. and Fox S. H., 1949, J. Biol. Chem., 178:517.
2. H. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopoeial Convention, Rockville, MD.

Revision :02 / 2019

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories 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 or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. 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.