



## B12 Assay Medium

MM036

B12 Assay Medium is recommended for microbiological assay of Vitamin B12 by using *Lactobacillus leichmannii* ATCC 7830 as the test organism in accordance with Indian pharmacopoeia.

### Composition\*\*

Ingredients	Gms / Litre
Acicase, vitamin free	10.000
Dextrose	40.000
Asparagine	0.200
Sodium acetate	20.000
Ascorbic acid	4.000
L-Cystine	0.400
L-Tryptophan	0.200
Adenine sulphate	0.020
Uracil	0.020
Xanthine	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 dihydrochloride	0.0008
Folic acid	0.0002
Potassium dihydrogen phosphate	1.000
Dipotassium hydrogen 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)	5.50-6.00

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 7.94 grams in 100 ml 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 (2),

AOAC (3) and IP (4) using *Lactobacillus leichmannii* ATCC 7830 as the test organism.

Standard curve is constructed with known dilutions of vitamin B12 standards (2, 3,4 ).

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

## 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 7.9 % w/v aqueous solution at 25°C. pH : 5.50-6.00

### pH

5.50 - 6.00

### Growth

Gradual increase in growth with increasing 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

MM036: 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 below 8°C in tightly closed container, preferably in dessicators and use freshly prepared medium. Use before expiry date on the label.

## Reference

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 Pharmacopoeial 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
4. Indian Pharmacopoeia, 2018, Ministry of Health and Family Welfare, Govt. of India

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