

TM 1985 – B12 ASSAY MEDIUM (using *L.leichmannii*)

INTENDED USE

For microbiological assay of Vitamin B12 by using *Lactobacillus leichmannii*.

PRODUCT SUMMARY AND EXPLANATION

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. It was first described by Capp et al and is recommended by USP and AOAC, using *Lactobacillus leichmannii* ATCC 7830 as the test organism.

Standard curve is constructed with known dilutions of vitamin B12 standards. Inoculum for the assay is prepared by sub-culturing 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.

COMPOSITION

Ingredients	Gms / Ltr
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

PRINCIPLE

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. Phosphates present provide ions to the medium. Glucose acts as a source of energy and agar acts as a solidifying agent.

INSTRUCTION FOR USE

- Dissolve 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 psi 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 SPECIFICATIONS

- Appearance of Powder** : Cream to yellow homogeneous having a tendency to form soft lumps which can be easily broken down to powder form.
- Appearance of prepared medium** : Light amber coloured clear solution that may contain a slight precipitate.
- pH (at 25°C)** : 6.1±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
<i>Lactobacillus leichmannii</i>	7830	50-100	Vitamin B12 assays passes	30°C-35°C	16-24 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE



Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

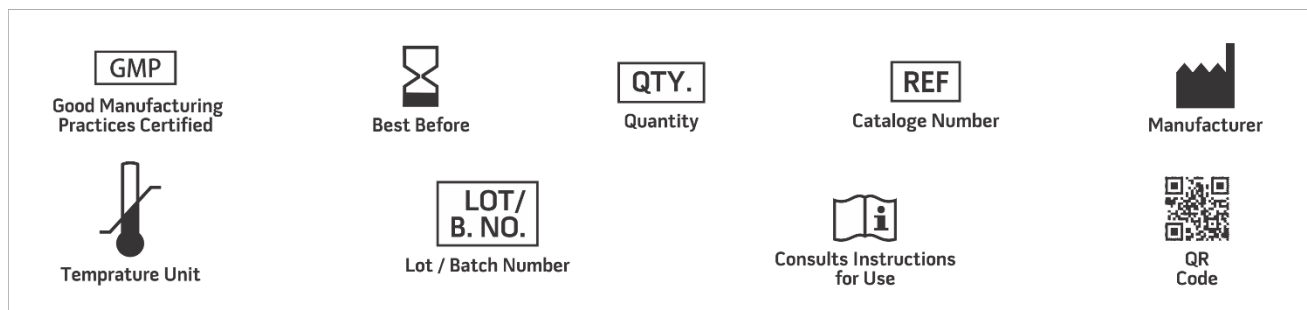
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopeial Convention, Rockville, MD.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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