# TM 1866 – B12 ASSAY AGAR (using L.leichmannii)

## INTENDED USE

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

## 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.

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 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 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.

## 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

The medium contains dextrose which acts as a source of energy. The phosphates ions in the medium helps in buffering the medium. Sodium chloride present helps in maintain the osmotic balance.

## 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.

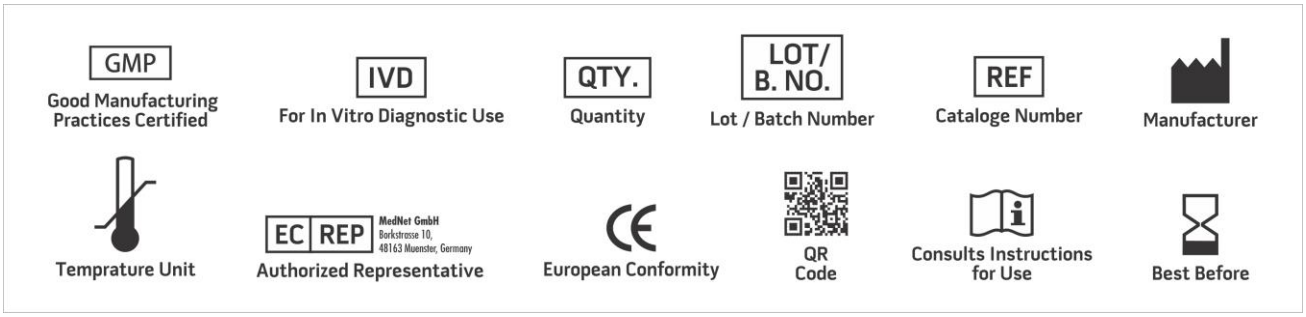
| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Incubation Temperature | Incubation Period |
|---|---|---|---|---|---|
| *Lactobacillus leichmannii* | 7830 | 50-100 | Gradual increase in growth with increasing USP Cyanocobalamin reference standard levels of 0.0, 0.025, 0.050, 0.075, 0.1, 0.125, 0.150 and 0.2 ng per assay tube is recorded as equivalent increase in absorbance at 620nm. | 35-37°C | 18-24 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. 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.
4. 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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