B12 Inoculum Broth

M206

Intended Use:

Recommended for preparing the inoculum of *Lactobacillus leichmannii* ATCC 7830 for the microbiological assay of Vitamin B12.

Composition**

Ingredients	Gms / Litre
Proteose peptone	7.500
Yeast extract	7.500
Dextrose (Glucose)	10.000
Polysorbate 80 (Tween 80)	0.100
Potassium dihydrogen phosphate	2.000
Tomato juice (from 100 ml)	5.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.10 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes or flasks as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

This medium which is rich in nutrients, is recommended by USP for inoculum preparation of *Lactobacillus leichmannii* ATCC 7830, the test bacterium used in microbiological estimation of Vitamin B12(5). *Lactobacillus* species have very exacting nutritional requirements for amino acids and vitamins. This restricts them to nutritionally compete in the environment.

Lactobacillus species grow poorly on non-selective media. Kulp (3) found that the growth of *Lactobacillus acidophilus* was enhanced with tomato juice, while investigating the use of tomato juice on bacterial development, which was reported earlier by Mickle and Breed (4) for the microbiological assay of vitamins.

Proteose peptone serves as a source of nitrogen and amino acids. Yeast extract is the vitamin source. Tomato juice is added to create the proper acidic environment. Dextrose is the carbon source and Polysorbate 80 acts as an emulsifier. Potassium dihydrogen phosphate provides buffering capacity.

For preparing inoculum, the culture is grown in 5 ml sterile B12 Inoculum Broth for 18 to 24 hours at 35°C and then the culture is centrifuged to obtain cell sediment. The supernatant is decanted and the cells are suspended in B12 Assay Medium (M036). This cell suspension is used as an inoculum after adjusting its density.

Type of specimen

Pure isolates

Specimen Collection and Handling:

For preparing inoculum, the culture is grown in 5 ml sterile B12 Inoculum Broth for 18 to 24 hours at 35°C and then the culture is centrifuged to obtain cell sediment. The supernatant is decanted and the cells are suspended in B12 Assay Medium (M036). This cell suspension is used as an inoculum after adjusting its density.

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 guidleines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Freshly prepared plates must be used or it may result in erroneous results.

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 free flowing powder

Colour and Clarity of prepared medium

Amber coloured, clear to slightly opalescent solution in tubes

Reaction

Reaction of 3.2% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)
Lactobacillus leichmannii ATCC 7830	50-100

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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. Use before expiry date on the label.
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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. 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.
3. Kulp and White, 1932, Science 76:17.
4. Mickle and Breed, 1925, Technical Bulletin 110, NY State Agriculture Ex. station, Geneva, N. Y.
5. The United States Pharmacopoeia, 2006, USP 29/ NF 24, The United States Pharmacopoeial Covention, Rockville, MD.

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