



## B12 Inoculum Broth (*Lactobacillus delbrueckii* subsp. *Lactis*)

MU206

B12 Inoculum Broth is used for preparing the inoculum of *Lactobacillus delbrueckii* subsp. *Lactis* ATCC 7830 in the microbiological assay of Vitamin B12.

### Composition\*\*

Ingredients	g/ L
Peptone	7.500
Yeast extract	7.500
Dextrose	10.000
Monopotassium 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. gram in 1000 ml distilled water. Add 10 ml of Polysorbate 80. Heat if necessary to dissolve the medium completely. Distribute in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

### Principle And Interpretation

This medium which is rich in nutrients, is recommended by USP (1) for inoculum preparation of *Lactobacillus delbrueckii* subsp. *Lactis* ATCC 7830, the test bacterium used in microbiological estimation of Vitamin B12. *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 (2) 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 (3) for the microbiological assay of vitamins.

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. Monopotassium phosphate provides buffering capacity.

For preparing inoculum, the culture is grown in 10 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. 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. 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 guidelines 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

MU206: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth
<b>Cultural Response</b> <i>\$Lactobacillus delbrueckii</i> subsp. <i>Lactis</i> ATCC 7830	50-100	good-luxuriant

Key: \$ formly known as *Lactobacillus leichmannii*.

## 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 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

## Reference

1. The United States Pharmacopoeia, 2022, The United States Pharmacopoeial Convention, Rockville, MD.
2. Kulp and White, 1932, Science 76:17.
3. Mickle and Breed, 1925, Technical Bulletin 110, NY State Agriculture Ex. station, Geneva, N. Y.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.

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### Disclaimer :

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