



## B12 Inoculum Broth (*L. leichmannii*)

M206

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

### Composition\*\*

Ingredients	Gms / Litre
Proteose peptone	7.500
Yeast extract	7.500
Dextrose	10.000
Polysorbate 80	0.100
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 32.1 grams in 1000 ml distilled water. 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 for inoculum preparation of *Lactobacillus leichmannii* 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.

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. Monopotassium 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.

### 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

M206: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)
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Cultural Response

*Lactobacillus leichmannii* 50-100  
ATCC 7830

### Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

### Reference

1. The United States Pharmacopoeia, 2006, USP 29/ NF 24, 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.

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### Disclaimer :

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