

PRODUCT SPECIFICATION SHEET

B12 Inoculum Broth (DM029)

Intended Use

B12 Inoculum Broth (DM029) is recommended for inoculum preparation of *Lactobacillus leichmannii* ATCC 7830, for Vitamin B12 microbiological assay.

Product Summary and Explanation

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⁽¹⁾ 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⁽²⁾ for the microbiological assay of vitamins.

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose: Maintenance Media for carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose; Inoculum Media to condition the test culture for immediate use; and Assay Media to permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

B12 Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 in the microbiological assay of vitamin B12 according to the USP.⁽³⁾

Principles of the Procedure

B12 Inoculum Broth contains peptic digest of animal tissue which 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.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	7.50
Yeast extract	7.50
Dextrose	10.00
Monopotassium phosphate	2.00
Tomato juice (from 100 ml)	5.00
Polysorbate 80	0.10
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. To avoid contamination of media or glassware in microbiological assay procedures great care must be taken. Glassware used should be scrupulously clean and free from detergents and other chemicals.
4. Extremely small amounts of foreign material may be sufficient to give erroneous results.

Directions

1. Suspend 32.10 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense desired amounts in tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

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Dehydrated Appearance	Cream to yellow coloured homogeneous free flowing powder
Prepared Medium	Amber coloured, clear to slightly opalescent solution in tubes
Reaction of 3.2% solution	pH 6.8 \pm 0.2 at 25°C
Gel Strength	Not Applicable

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

Sr. No.	Organisms	Inoculum (CFU)
1.	<i>Lactobacillus leichmannii</i> ATCC 7830	50-100

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

- Preparation of Inoculum
 - For preparing inoculum, the culture is grown in 5 ml sterile B12 Inoculum Broth for 18 to 24 hours at 35°C.
 - Culture is centrifuged to obtain cell sediment. The supernatant is decanted and the cells are suspended in B12 Assay Medium (DM269).
 - After adjusting the density, this cell suspension is used as an inoculum.
- Refer to appropriate procedures outlined in the USP, for a complete discussion of vitamin assay methodology.

Results

Refer to appropriate references and specific test procedures.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

- The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
- All conditions of the assay must be followed precisely, for successful results of these procedures.
- Aseptic technique should be used throughout the assay procedure.
- The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.

Packaging

Product Name : B12 Inoculum Broth

Product Code : DM029

Available Pack sizes : 100gm

References

- Mickle and Breed, 1925, Technical Bulletin 110, NY State Agriculture Ex. station, Geneva, N.Y.
- Kulp and White, 1932, Science 76:17.
- The United States Pharmacopoeia, 2006, USP 29/ NF 24, The United States Pharmacopoeial Convention, Rockville, MD.

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Further Information

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED

Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall', Thane (W) - 400607. M.S. INDIA.
Ph: +91-9320126789/9833630009/9819991103
Email: sales@micromasterlab.com

DM029PSS, QAD/FR/024, Rev.00

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