



PRODUCT SPECIFICATION SHEET

B12 Culture Agar (*L. leichmannii* Maintenance Medium) (DM028)

Intended Use

B12 Culture Agar (*L. leichmannii* Maintenance Medium) (DM028) is recommended for propagation, cultivation and maintenance of *Lactobacillus leichmannii* ATCC 7830.

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 quantification 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 Culture Agar recommended by USP for cultivation and maintenance of *Lactobacillus leichmannii* ATCC 7830 (*Lactobacillus delbrueckii subsp. lactis* ATCC 7830) which is used as a test bacterium during the microbiological estimation of vitamin B12.⁽³⁾

Principles of the Procedure

B₁₂ Culture Agar (*L. leichmannii* Maintenance Medium) 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
Peptic digest of animal tissue	7.50
Yeast extract	7.50
Dextrose	10.00
Monopotassium phosphate	2.00
Tomato juice (from 100 ml)	5.00
Polysorbate 80	0.10
Agar	10.00
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 42.10 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense 10 ml amounts in tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubed medium in an upright position with rapidity to avoid colour formation due to overheating.





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Quality Control Specifications

Dehydrated Appearance	Cream to yellow coloured homogeneous free flowing powder
Prepared Medium	Medium amber coloured, clear to slightly opalescent gel forms in tubes
Reaction of 4.21% solution	pH 6.8 + 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Lactobacillus leichmannii</i> ATCC 7830	50-100	good-luxuriant

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

Test Procedure

Preparation and Maintenance of stock

1. Prepare the stock cultures of *Lactobacillus leichmannii* ATCC 7830 by stab inoculation of 3 or more tubes.
 2. These stab cultures are made at least 3 times in a week. Do not use the culture for preparing assay inoculum if it is over 4 days old.
 3. Make at least 10 successive transfers of the culture in 15 days period, before using a fresh culture for assay.
 4. Incubate the culture for 16-24 hours at 35°C but hold constant within 0.5°C. After incubation, store at 2-8°C.
- 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

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. All conditions of the assay must be followed precisely, for successful results of these procedures.
3. Aseptic technique should be used throughout the assay procedure.
4. 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 Culture Agar (*L. leichmannii* Maintenance Medium)

Product Code : DM028

Available Pack sizes : 100gm

References

1. Mickle and Breed, 1925, Technical Bulletin 110, NY State Agriculture Ex. station, Geneva, N.Y.
2. Kulp and White, 1932, Science 76:17.
3. 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.



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DM028PI, Rev.0,

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