PRODUCT SPECIFICATION SHEET

Lactobacilli Broth, AOAC (DM131)

Intended Use
Lactobacilli Broth, AOAC (DM131) is recommended for inoculum preparation of the test bacteria used in microbiological assays of B vitamins.

Product Summary and Explanation
Lactobacillus species grow poorly on non-selective culture media and require special nutrients for their growth. Vitamin assay media are prepared for use in 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. Mickle and Breed[1] reported the use of tomato juice in culture media for lactobacilli. Kulp and White,[2] while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.
Lactobacilli Broth, AOAC was formulated by Loy[3] and recommended by AOAC[4] for preparing inocula of test bacteria used for Microbiological assay of Vitamin B. Stock cultures of *Lactobacillus leichmanni ATCC 7830*, *Lactobacillus plantarum ATCC 8014*, *Lactobacillus casei ATCC 7469*, *Enterococcus hirae ATCC 8043* and other such B vitamin requiring strains.

Principles of the Procedure
Lactobacilli Broth AOAC contains peptonized milk and yeast extract which provides nitrogen, amino acids and vitamin sources essential for growth of organisms. Dextrose is the carbon and energy source. Monopotassium phosphate provides buffering system while tomato juice helps in lowering the pH thereby creating an acidic environment. Polysorbate 80 acts as an emulsifier.

Formula / Liter

Ingredients	Gms / Liter
Peptonized milk	15.00
Yeast extract	5.00
Dextrose	10.00
Tomato juice (100 ml)	5.00
Monopotassium phosphate	2.00
Polysorbate 80	1.00
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 used for microbiological assay procedures, great care must be taken.
4. To give erroneous results, extremely small amounts of foreign material may be sufficient.
5. Scrupulously clean glassware free from detergents and other chemical must be used.
6. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
7. Take precautions to keep sterilization and cooling conditions uniform.

Directions
1. Suspend 38 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.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium amber coloured clear solution in tubes
Reaction of 3.8% solution	pH 6.8 + 0.2 at 25oC
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Enterococcus hirae ATCC 8043*	50-100	good- luxuriant
2.	*Lactobacillus casei ATCC 7469*	50-100	good- luxuriant
3.	*Lactobacillus leichmannii ATCC 7830*	50-100	good- luxuriant
4.	*Lactobacillus plantarum ATCC 8014*	50-100	good- luxuriant

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

Test Procedure

Refer to appropriate procedures outlined in the references, for a complete discussion on vitamin assay methodology.

Inoculum

From a 18-24 hour stock culture subculture into 10 mL Lactobacilli Broth AOAC.
Incubate at 35-37°C for 18-24 hours or as specified in specific assay procedures.
Centrifuge the culture and decant the supernatant. Resuspend cells in 10 mL of sterile 0.9% NaCl solution or sterile single-strength basal assay medium.
Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise specified.
Dilute the washed suspension 1:100 with sterile 0.9% NaCl or sterile single-strength basal assay medium or as specified.
Inoculum concentration should be adjusted according to limits specified in the references.

Results

Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 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. Before using a culture in any assay, at least 2 successive transfers during a 1-2 week period are essential. Any culture older than one week should not be used.
2. Test organism used for inoculating an assay medium must be cultured and maintained on media recommended for that purpose.
3. Aseptic technique should be followed throughout the vitamin assay procedure.
4. The use of altered or deficient media may result in mutants with different nutritional requirements that will not give a satisfactory response.
5. All conditions of the assay must be adhered to meticulously, for a successful completion of these procedures.

Packaging

Product Name : Lactobacilli Broth, AOAC
Product Code : DM131
Available Pack sizes : 500gm

References

1. Mickle and Breed. 1925. Technical Bulletin 110, N.Y. State Agriculture Ex. Station, Geneva, N.Y.
2. Kulp and White. 1932. Science 76:17.
3. Loy. 1958. J. Assoc. Off. Agri. Chem. 4:61.
4. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

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

DM131PSS,QAD/FR/024,Rev.00