



PRODUCT SPECIFICATION SHEET

Lactobacilli Agar, AOAC (DM130)

Intended Use

Lactobacilli Agar, AOAC (DM130) is recommended for carrying the stock cultures used in the microbiological assays of the 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 Agar, AOAC was formulated by Loy⁽³⁾ and recommended by AOAC⁽⁴⁾ for carrying stock cultures used for Microbiological assay of Vitamin B. Lactobacilli Agar, AOAC is used for the cultivation and maintenance of stock cultures of *Lactobacillus casei* ATCC 7469, *Lactobacillus fermentum* ATCC 9338, *Lactobacillus leichmannii* ATCC 4797, and *Lactobacillus viridescens* ATCC 12706.

Principles of the Procedure

Lactobacilli Agar 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
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 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 48 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.





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5. Cool the tubed agar medium in an upright position.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium amber coloured clear to slightly opalescent gel forms in tubes as butts
Reaction of 4.8% 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 18-48 hours.

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

Stock Cultures

1. Prepare stock cultures in one or more tubes of sterile Lactobacilli Agar AOAC.
2. Inoculate the medium using an inoculating needle.
3. Incubate at 30-40°C for 18-24 hours.
4. Store at 2-8°C.
5. Transfer at weekly or twice monthly intervals.

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 Agar, AOAC

Product Code : DM130

Available Pack sizes : 100gm





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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.



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