Lactobacilli Agar AOAC • Lactobacilli Broth AOAC

Intended Use

Lactobacilli Agar AOAC is used for maintaining stock cultures for microbiological assays of vitamins and amino acids.

Lactobacilli Broth AOAC is used for preparing inocula for microbiological assays of vitamins and amino acids.

Summary and Explanation

Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. 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.

Lactobacilli Agar AOAC[1] and Lactobacilli Broth AOAC[1] are prepared according to the formula recommended by Loy.[2] Lactobacilli Agar AOAC is used for maintaining stock cultures. Lactobacilli Broth AOAC is used to prepare inocula of *Lactobacillus delbruecki* subsp. *lactis (Lactobacillus leichmannii)* ATCC™ 7830, *Enterococcus hirae* ATCC 8043, *Lactobacillus plantarum* ATCC 8014, *Lactobacillus (casei) rhamnosus* ATCC 7469 and other organisms used in the microbiological assay of B vitamins.

Lactobacillus species grow poorly on nonselective culture media and require special nutrients. Mickle and Breed[3] reported the use of tomato juice in culture media for lactobacilli. Kulp and White,[4] while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

User Quality Control

Identity Specifications

Difco™ Lactobacilli Agar AOAC

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in purified water upon boiling 2-3 minutes. Solution is medium amber, opalescent when hot, clearer when cooled to 45-50°C.
Prepared Appearance:	Medium amber, clear.
Reaction of 4.8% Solution at 25°C:	pH 6.8 ± 0.2

Difco™ Lactobacilli Broth AOAC

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling 2-3 minutes. Solution is medium amber, opalescent when hot, clear after cooling, may have a slight precipitate.
Prepared Appearance:	Medium amber, clear, may have a slight precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Lactobacilli Agar AOAC or Lactobacilli Broth AOAC

Prepare the medium per label directions. Inoculate Lactobacilli Agar AOAC by stabbing the medium with test organisms; incubate at 35 ± 2°C for 18-48 hours. Inoculate Lactobacilli Broth AOAC with test organisms and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Enterococcus hirae	8043	10^2-10^3	Good
Lactobacillus rhamnosus	7469	10^2-10^3	Good
Lactobacillus delbrueckii subsp. *lactis*	7830	10^2-10^3	Good
Lactobacillus plantarum	8014	10^2-10^3	Good

Principles of the Procedure

Peptonized milk and yeast extract provide the nitrogen, amino acids and vitamins sources in Lactobacilli Agar AOAC and Lactobacilli Broth AOAC. Dextrose is a carbon source to facilitate organism growth. Tomato juice creates the proper acidic environment. Monopotassium phosphate is a buffering agent. Polysorbate 80 acts as an emulsifier. Agar is the solidifying agent in Lactobacilli Agar AOAC.

Formulae

Difco™ Lactobacilli Agar AOAC

Approximate Formula* Per Liter

Peptonized Milk	15.0	g
Yeast Extract	5.0	g
Dextrose	10.0	g
Tomato Juice (from 100 mL)	5.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	1.0	g
Agar	10.0	g

Difco™ Lactobacilli Broth AOAC

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Precautions

Great care must be taken to avoid contamination of media or glassware used for microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemical must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform.

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ Lactobacilli Agar AOAC – 48 g;
   Difco™ Lactobacilli Broth AOAC – 38 g.
   Mix thoroughly.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

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 30-37°C for 18-24 hours.
4. Store at 2-8°C.
5. Transfer at weekly or twice monthly intervals.

Inoculum

1. Subculture from a 16-24 hour stock culture into 10 mL Lactobacilli Broth AOAC.
2. Incubate at 35-37°C for 16-24 hours or as specified in specific assay procedures.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 mL of sterile 0.9% NaCl solution or sterile single-strength basal assay medium.
5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% NaCl or sterile single-strength basal assay medium or as indicated. Where applicable, inoculum concentration should be adjusted according to limits specified in the references.[1,5]

For a complete discussion on vitamin assay methodology refer to appropriate procedures outlined in the references.[1,5]

Expected Results

Refer to appropriate references for vitamin assay results.[1,5]

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for that purpose.
2. Aseptic technique should be used throughout the vitamin assay procedure.
3. The use of altered or deficient media may result in mutants with different nutritional requirements that will not give a satisfactory response.
4. For a successful completion of these procedures, all conditions of the assay must be adhered to meticulously.

References

1. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
2. Loy. 1958. J. Assoc. Off. Agri. Chem. 4:61.
3. Mickle and Breed. 1925. Technical Bulletin 110, N.Y. State Agriculture Ex. Station, Geneva, N.Y.
4. Kulp and White. 1932. Science 76:17.
5. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26 Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Lactobacilli Agar AOAC

AOAC

Cat. No. 290010 Dehydrated – 100 g*

Difco™ Lactobacilli Broth AOAC

AOAC

Cat. No. 290110 Dehydrated – 100 g*

**Store at 2-8°C.*