Micro Assay Culture Agar • Micro Inoculum Broth

Intended Use

Micro Assay Culture Agar is used for cultivating lactobacilli and other organisms used in microbiological assays.

Micro Inoculum Broth is used for preparing the inoculum of lactobacilli and other microorganisms used in 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 carrying 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.

Micro Assay Culture Agar is used for maintaining stock cultures of lactobacilli and other test microorganisms. This medium is also used for general cultivation of lactobacilli.

Micro Inoculum Broth is used for cultivating lactobacilli and preparing the inoculum for microbiological assays.

Principles of the Procedure

Peptone provides nitrogen and amino acids in both Micro Assay Culture Agar and Micro Inoculum Broth. Yeast extract is a vitamin source. Dextrose is a carbon source. Monopotassium phosphate is a buffering agent. Polysorbate 80 acts as an emulsifier. Agar is the solidifying agent (Micro Assay Culture Agar).

User Quality Control

Identity Specifications

Difco™ Micro Assay Culture Agar

Dehydrated Appearance:	Light tan to tan, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 6.7 ± 0.2

Difco™ Micro Inoculum Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent, without precipitate.
Reaction of 3.7% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Micro Assay Culture Agar or Micro Inoculum Broth

Prepare the medium per label directions. Inoculate with test organisms. Incubate Micro Assay Culture Agar at 35 ± 2°C for 18-48 hours; incubate Micro Inoculum Broth at 35-37°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

Formulae

Difco™ Micro Assay Culture Agar

Approximate Formula* Per Liter

Proteose Peptone No. 3	5.0	g
Yeast Extract	20.0	g
Dextrose	10.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	0.1	g
Agar	10.0	g

Difco™ Micro Inoculum Broth

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 in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals 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 throughout the assay.

Directions for Preparation from Dehydrated Product

Difco™ Micro Assay Culture Agar

1. Suspend 47 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense 10 mL amounts into 16-20 mm diameter tubes.
4. Autoclave at 121°C for 15 minutes.
5. Agitate tubes prior to solidification to disperse the flocculent precipitate.
6. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Micro Inoculum Broth

1. Dissolve 37 g of the powder in 1 L of purified water.
2. Dispense 10 mL quantities into tubes of 16-20 mm diameter.
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 triplicate on Micro Assay Culture Agar, inoculating tubes using a straight-wire inoculating needle.
2. Incubate tubes at 30-37°C for 18-24 hours.
3. Store at 2-8°C.
4. Transfer cultures at weekly or twice-monthly intervals.

Assay Inoculum

1. Subculture from a 16-24 hour stock culture of lactobacilli in Micro Assay Culture Agar into a 10 mL tube of Micro Inoculum Broth.
2. Incubate at 35-37°C for 16-24 hours or as specified in the assay procedure.
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% single strength basal assay medium or as indicated. Where applicable, adjust inoculum concentration according to limits specified in the references.[1,2]

For a complete discussion of vitamin assay methodology, refer to appropriate procedures.[1,2]

Expected Results

For test results on vitamin assay procedures, refer to appropriate procedures.[1,2]

Limitations of the Procedure

1. Test organisms used in assay procedures must be cultured and maintained on media recommended for this purpose.
2. Follow assay directions exactly. The age, preparation and size of inoculum are extremely important factors in obtaining a satisfactory assay result.
3. Although other media and methods may be used successfully for maintaining cultures and preparing inocula, uniformly good results will be obtained if the methods described are followed exactly.
4. Aseptic technique should be used throughout the microbiological assay procedure.
5. The use of altered or deficient media may create mutants having different nutritional requirements. Such organisms will not produce a satisfactory test response.

References

1. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed, online. AOAC International, Gaithersburg, Md.
2. 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™ Micro Assay Culture Agar

Cat. No. 231920 Dehydrated – 500 g

Difco™ Micro Inoculum Broth

Cat. No. 211813 Dehydrated – 500 g