

Instructions for Use

LACTOBACILLI MRS AGAR

Cat. no. G117	Lactobacilli MRS Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G197	Lactobacilli MRS Agar, 15x60mm Plate, 12ml	10 plates/bag
Cat. no. G179	Lactobacilli MRS Agar with Cycloheximide, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. U204	Lactobacilli MRS Agar, 500ml Polycarbonate Bottle, 400ml	10 bottles/box

INTENDED USE

Hardy Diagnostics Lactobacilli MRS Agar is recommended for the isolation, enumeration and cultivation of *Lactobacillus* spp.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Lactobacilli MRS Agar was developed by researchers deMan, Rogosa and Sharpe as an alternative non-selective media for the cultivation of fastidious lactobacilli. Previous media for the cultivation of lactobacilli employed the use of tomato juice, however, tomato juice agar was undesirable because of its variability and difficulty in preparation. A media described by Rogosa, Mitchell and Wiseman, although adequate for most lactobacilli, was found to be unsatisfactory for use with some dairy lactobacilli organisms. With this in mind, deMan, Rogosa and Sharpe hoped to develop a new and general non-selective growth medium for lactobacilli. They found that the inclusion of Tween® 80, citrate and acetate resulted in improved growth for lactobacilli, while citrate and acetate weakly inhibit the growth of gram negative bacilli and fungi. Manganese and magnesium are inorganic ions necessary for growth in the presence of citrate.⁽¹⁾ This media shows a low degree of selectivity; therefore, secondary accompanying microflora may grow well and compete for nutrients. However, most of these accompanying microorganisms can be inhibited by the addition of various concentrations of selective agents, such as cycloheximide, polymyxin, thallium acetate, sorbic acid, acetic acid, or sodium nitrite to the medium. Lactobacilli MRS Agar with Cycloheximide is useful for inhibiting fungi that may be in the sample.

Lactobacilli are long, slender, non-sporeforming, gram-positive rods that are generally facultatively anaerobic, most of which grow well with reduced oxygen tension and increased CO₂.⁽²⁾ Lactobacilli are important microorganisms for the dairy, food, and beverage industry. Microbial spoilage of fruit juice is most commonly due to aciduric organisms such as lactic acid bacteria and yeast.⁽³⁾ Lactic acid organisms are important to the dairy industry for determining the cause of acid defects in dairy products as well as in evaluating the lactic starter cultures in cured cheese and cultured milks.⁽⁴⁾ *Lactobacillus brevis* is a contaminating organism in the production of beer that, if present, can be responsible for its spoilage.⁽⁶⁾ These lactobacilli damage beer by causing turbidity and a poor flavor due to diacetyl, a strongly flavored by-product of their metabolism.⁽⁵⁾ Finally, lactobacilli are used by the vegetable food industry for the fermentation of cabbage to sauerkraut.⁽⁵⁾

FORMULA

Ingredients per liter of deionized water:*

Dextrose	20.0gm
Peptic Digest of Animal Tissue	10.0gm
Beef Extract	10.0gm
Yeast Extract	5.0gm
Sodium Acetate	5.0gm
Dipotassium Phosphate	2.0gm
Ammonium Citrate	2.0gm
Tween [®] 80	1.0gm
Magnesium Sulfate	0.1gm
Manganese Sulfate	0.05gm
Agar	15.0gm

In addition, Lactobacilli MRS Agar with Cycloheximide contains the following ingredient per liter:*

Cycloheximide	3mg
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Final pH 6.5 +/- 0.3 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Hardy Diagnostics Lactobacilli MRS Agar can be used in several ways. These plates can be streaked directly or used with a membrane filter for plate counts. Lactobacilli MRS Agar plates are incubated at 35°C. for 24-72 hours in a CO₂ incubator or under microaerophilic conditions (5% carbon dioxide, 5-10% oxygen). Lower temperatures of 22-25°C. may be used for psychrotrophic counts or 42°C. for thermophilic counts.

It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

For re-melting solid tube and bottle media: Autoclave containers with slightly loose caps at 121°C for 1-3 minutes or until melted. Do not heat media longer than 3 hours at 45-50°C. Alternatively, solid agar in capped containers can be racked and placed in a covered, boiling water bath (100°C) before use. There should be enough water in the water bath to reach the top of the media line. A covered water bath will maintain consistent temperature of the media until melted. Cool media to 45-50°C and aseptically dispense into sterile containers. **Note:** Sterile solidified media can be re-melted only once. In addition, the use of microwaves to melt media is not advised.

INTERPRETATION OF RESULTS

Lactobacilli appear as large clear colonies after 24-72 hours incubation at 35°C. in an enhanced CO₂ environment.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Lactobacilli MRS Agar has a low degree of selectivity allowing for the growth of other lactic acid organisms such as *Pediococcus* and *Leuconostoc* species. Presumptive *Lactobacillus* colonies can be subcultured to Hardy Diagnostics Lactobacilli MRS Broth for further identification.

Lactobacilli MRS Agar with cycloheximide can be used to inhibit the overgrowth of accompanying microbial flora such as yeasts and molds.

It is important to maintain the appropriate moisture content of the plates during incubation. Do not allow the surface of the plates to dry out as this will inhibit the growth of lactobacilli due to an increasing concentration of acetate on the agar surface.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

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Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Lactobacillus delbrueckii</i> ATCC® 7830	A	24-72hr	35°C	CO ₂ **	Growth; clear colonies
<i>Lactobacillus fermentum</i> ATCC® 9338	A	24-72hr	35°C	CO ₂ **	Growth; clear colonies
<i>Lactobacillus acidophilus</i> ATCC® 4356	A	24-72hr	35°C	CO ₂ **	Growth; clear colonies
Lactobacilli MRS Agar with Cycloheximide is also tested with the following organisms:					
<i>Candida albicans</i> ATCC® 10231	A	24-72hr	35°C	CO ₂ **	Growth

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

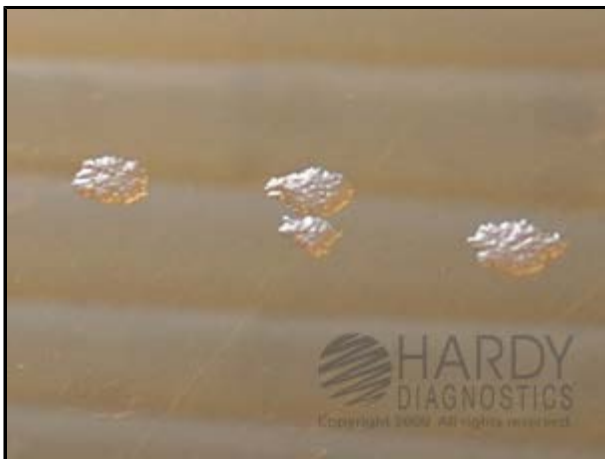
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Lactobacilli MRS Agar should appear slightly opalescent, and medium amber in color.

Lactobacilli MRS Agar with Cycloheximide should appear slightly opalescent, and light to medium amber in color.



Lactobacillus acidophilus (ATCC® 4356) colonies growing on Lactobacilli MRS Agar (Cat. no. G117). Incubated in CO₂ for 72 hours at 35°C.



Uninoculated plate of Lactobacilli MRS Agar (Cat. no. G117).

REFERENCES

1. De Man, J.C., Rogosa, M., Sharpe, M. Elisabeth. 1960. A Medium for the Cultivation of Lactobacilli. *J. Appl. Bact.*;

23:130-135.

2. MacFaddin, Jean F. *Biochemical Tests for Identification of Medical Bacteria*, Lippincott Williams & Wilkins, Philadelphia, PA.
3. Vanderzant, Carl., and Splittstoesser, Don F. 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. American Public Health Association, Washington, D.C.
4. Marshall, Robert T. 1992. *Standard Methods for the Examination of Dairy Products*, 16th ed. American Public Health Association, Washington, D.C.
5. Doyle, Michael P., Beuchat, Larry R., and Montville, Thomas J. 1997. *Food Microbiology Fundamentals and Frontiers*, 1st ed. American Society for Microbiology, Washington, D.C.
6. Yasui, T., and Yoda, Y. 1997. Imaging of *Lactobacillus brevis* Single Cells and Microcolonies with a Microscope by an Ultrasensitive Chemiluminescent Enzyme Immunoassay with a Photon-counting Television Camera. *Appl. Environ. Microbiol.*; 63:4528-4533.
7. Association of Official Analytical Chemists. *Official Methods of Analysissm*, AOAC, Washington, D.C.
8. Committee of Revision for The United States Pharmacopeia. 2000. *The United States Pharmacopeia*, 24th rev. United States Pharmacopeial Convention, Rockville, MD.
9. American Public Health Association. 1992.
10. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>.

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