

Instructions for Use

LACTOBACILLI MRS BROTH

Cat. no. K15	Lactobacilli MRS Broth, 16x125mm Tube, 9ml	20 tubes/box
Cat. no. K17	Lactobacilli MRS Broth, 16x125mm Tube with Durham Tube, 9ml	20 tubes/box
Cat. no. U203	Lactobacilli MRS Broth, 500ml Polycarbonate Bottle, 500ml	10 bottles/box
Cat. no. U314	Lactobacilli MRS Broth, 125ml Polycarbonate Bottle, 99ml	12 bottles/box

INTENDED USE

Hardy Diagnostics Lactobacilli MRS Broth 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

MRS Broth was developed by deMan, et al., to provide luxuriant growth of lactobacilli isolated from oral, fecal, and other specimen sources.⁽⁶⁾ Lactobacilli MRS Broth is an improved medium for lactobacilli, it supports good growth and is particularly useful for a number of fastidious strains which grow poorly on other general media.

Peptone and dextrose provide the carbon, nitrogen and other elements necessary for growth. Tween[®] 80, acetate, magnesium and manganese provide growth factors needed for culturing a variety of lactobacilli. Those ingredients may also inhibit the growth of some organisms other than lactobacilli. In addition, tomato juice is not required. In a slightly modified form, it can also be used as a basal medium for fermentation tests.

FORMULA

Ingredients per liter deionized water:*

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

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 contamination, deterioration, discoloration, 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

1. Samples can be inoculated directly into Lactobacilli MRS Broth.
2. Incubate broth tubes at 35°C. for 3 days, or at 30°C. for 5 days, in an aerobic atmosphere.
3. Subculture growth in broth tubes to an appropriate solid media.

INTERPRETATION OF RESULTS

Growth of Lactobacilli will produce turbidity in Lactobacilli MRS Broth. Growth may be subcultured onto the appropriate media for use in additional procedures. Refer to appropriate references for recommendations on the culture of *Lactobacillus* spp.⁽¹⁻⁵⁾

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.

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:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Lactobacilli MRS Broth with Durham Tube:					
Lactobacillus fermentum ATCC® 9338	A	18-24hr	35°C	Aerobic	Growth; gas in durham tube
Lactobacillus acidophilus ATCC® 4356	A	1-7 days	35°C	Aerobic	Growth; no gas in durham tube
Lactobacilli MRS Broth: U203					
Lactobacillus acidophilus ATCC® 4356	A	1-7 days	35°C	Aerobic	Growth
Weissella paramesenteroides ATCC® 33313	A	1-7 days	35°C	Aerobic	Growth
Lactobacilli MRS Broth: U314					
Lactobacillus acidophilus ATCC® 4356	A	18-24 hours	35°C	Aerobic	Growth
Escherichia coli ATCC® 25922	A	18-24 hours	35°C	Aerobic	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 Broth should appear clear, with a very slight opalescence, and dark amber in color.



Lactobacillus fermentum (ATCC® 9338) growing in Lactobacilli MRS Broth (Cat. no. K17). The bubble in the Durham tube was indicative of gas production. Incubated aerobically for 24 hours at 35°C.



Lactobacillus acidophilus (ATCC® 4356) growing in Lactobacilli MRS Broth (Cat. no. K17). Incubated aerobically for 24 hours at 35°C.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, 12th ed. C.V. Mosby Company, St. Louis, MO.
3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
6. deMan, Rogosa and Sharpe. 1960. *Journal of Applied Bacteriology*; 23(1) 130-135.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

ATCC is a registered trademark of the American Type Culture Collection.

Tween is a registered trademark of ICI Americas, Inc.

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