

Instructions for Use

CRITERION[™] LACTOBACILLUS SELECTIVE (LBS) AGAR BASE

Cat. no. C6050	CRITERION TM Lactobacillus Selective (LBS) Agar Base	168gm
Cat. no. C6051	CRITERION [™] Lactobacillus Selective (LBS) Agar Base	500gm
Cat. no. C6052	CRITERION [™] Lactobacillus Selective (LBS) Agar Base	2kg
Cat. no. C6053	CRITERION TM Lactobacillus Selective (LBS) Agar Base	10kg
Cat. no. C6054	CRITERION TM Lactobacillus Selective (LBS) Agar Base	50kg

INTENDED USE

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Hardy Diagnostics CRITERION[™] Lactobacillus Selective Agar Base is recommended as a general purpose growth medium for the selective isolation, cultivation, and enumeration of lactobacilli.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

Lactobacilli (LAB) are gram-positive, non-sporing, bacilli that microscopically appear in chains varying from long, slender, and sometimes bent, to short and often coryneform-like. Pigment is rarely produced, but if present colonies can range from yellow, orange to rust, or brick in color. As lactobacilli are mostly microaerophilic, surface growth is enhanced by anaerobiosis or lowered oxygen pressure or enhanced CO_2 (5-10%). Lactobacilli grow best in acidic media with a pH range of 4.5-6.4 and exhibit a wide temperature range, from 2 to 53°C, but prefer temperatures from 30 to 40°C.^(2,4) Lactobacilli are part of the normal flora in the mouth, intestinal tract and vagina of animals. Aside from dental caries, they are rarely considered pathogenic. They are important to the food, dairy and beverage industries both in the production and spoilage of products.⁽⁴⁾

Lactobacilli are significant contaminants in the food, dairy, and beverage industries. Although lactobacilli are found on plant surfaces in low numbers, they grow luxuriously in all decaying plants and especially fruits.⁽⁴⁾ The microbial spoilage of unpasteurized fruit juices is most often the result of lactobacilli. Due to their preference for acidic environments, even citrus concentrates can be spoiled with acetylmethylcarbinol and diacetyl from lactobacilli that have flourished in the juice prior to high temperature evaporation.⁽⁵⁾ Lactobacilli are also contaminants in the meat industry acting as spoilage agents of fresh and canned meat, as well as raw marinated herring and diseased rainbow trout. Additionally, lactobacilli are associated with spoilage of beer and milk products.⁽⁴⁾

In direct contrast to their role as spoilage organisms, lactobacilli also aid in the production of a variety of food, beverage and dairy products. The production of lactic acid and gas, a sugar fermentation reaction performed by LAB, is utilized in the production of sausages, salami, yogurt, cheeses, kefir, acidophilus milk, production of sourdough bread, sauerkraut. Because lactobacilli are sensitive to most antibiotics active against gram-positive organisms, they are often used as a method to detect antibiotics in milk.

Hardy Diagnostics CRITERIONTM Lactobacillus Selective Agar Base is a selective agar for the isolation of lactobacilli. The media contains casein peptone and yeast extract for nutrients. Magnesium, manganese, ferrous sulfate and Tween[®] 80 are essential for most species of lactobacilli as they stimulate growth. Ammonium citrate, sodium acetate, and glacial acetic acid (to lower pH) are instrumental in the inhibitory nature of the media.^(3,4)

FORMULA

Gram weight per liter:	84.0gm/L
Sodium Acetate Hydrate	25.0gm
Dextrose	20.0gm
Pancreatic Digest of Casein	10.0gm
Monopotassium Phosphate	6.0gm
Yeast Extract	5.0gm
Ammonium Citrate	2.0gm
Tween [®] 80	1.0gm
Magnesium Sulfate	575.0mg
Manganese Sulfate	120.0mg
Ferrous Sulfate	34.0mg
Agar	15.0gm

Final pH 5.5 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-8°C. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not homogeneous, moist, and lumpy or if the color has changed from its original light beige.

Store the prepared culture media at 2-8°C.

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 "<u>Guidelines for Isolation</u> <u>Precautions</u>" 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

- 1. Suspend 84.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.
- 2. Add 1.32ml of glacial acetic acid.
- 3. Heat to boiling and mix to dissolve completely.
- 4. Sterilize in the autoclave at 121°C. for 15 minutes.
- 5. Cool to 45-50°C.
- 6. Dispense into sterile petri dishes.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., 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		Incubation		Results
	Method*	Time	Temperature	Atmosphere	

Lactobacillus acidophilus ATCC [®] 4356	А	24-48hr	35°C	CO ₂ **	Growth; small colonies
Lactobacillus plantarum ATCC [®] 8014	А	24-48hr	35°C	CO ₂ **	Growth; small colonies
Proteus hauseri ATCC [®] 13315	А	24-48hr	35°C	CO ₂ **	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Atmosphere of incubation is enriched with 5-10% CO₂.

USER QUALITY CONTROL

Users of dehydrated 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 <u>Certificate of Analysis</u> website. In addition, refer to the following document "<u>Finished Product</u> <u>Quality Control Procedures</u>," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERIONTM Lactobacillus Selective (LBS) Agar Base powder should appear homogeneous, moist, and lumpy, and light beige in color. The prepared media should appear clear with a slight opalescence, and light to medium amber in color.

REFERENCES

1. Doyle, M.P., Beuchat, L.R., and Montville, T.J. 1997. *Food Microbiology Fundamentals and Frontiers*, 1st ed. ASM Press, Washington, D.C.

2. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.

3. Marshall, R.T. 1993. *Standard Methods for the Examination of Dairy Products*, 16th ed. American Public Health Association, Washington, D.C.

4. Sneath, P.H.A., Nicholas, M.S., Sharpe, E.M., and Holt, J.G. 1986. *Bergey's Manual of Systematic Bacteriology*, Vol. II. Williams & Wilkins, Baltimore, MD.

5. Vanderzant, C. and Splittstoesser, D.F. 1992. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. American Public Health Association Washington, D.C.

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