

# LBS Agar • LBS Broth

## Intended Use

LBS (*Lactobacillus* Selection) Agar is used for the selective isolation and enumeration of lactobacilli.

LBS Broth is a selective medium for the isolation and cultivation of lactobacilli.

## Summary and Explanation

Rogosa et al. developed LBS Agar as a selective medium for the isolation and enumeration of oral and fecal lactobacilli.<sup>1,2</sup> They reported that the medium was more selective for lactobacilli than the tomato juice media previously employed and that the growth of molds, streptococci and spreading organisms was markedly reduced.

LBS Agar is used for selective recovery of lactobacilli from the oral cavity, especially tooth surfaces, intestinal flora,<sup>3,4</sup> the vagina,<sup>5</sup> meats and other foods<sup>6</sup> and dairy products.<sup>7</sup> Sabine and Vaselekos reported that incorporation of tomato juice resulted in a two-fold increase in the number of *L. acidophilus* organisms recovered from feces.<sup>8</sup>

LBS Broth is the fluid form of LBS Agar.<sup>1</sup> This broth medium may be used as a preliminary enrichment broth for detection of lactic acid organisms. Subcultures should be made to LBS Agar or to suitable general-purpose plating media.

## Principles of the Procedure

Peptone, yeast extract and dextrose supply nitrogenous and carbonaceous compounds for the support of bacterial growth. Polysorbate 80 is a source of growth factors, since it supplies fatty acids required for the metabolism of lactobacilli. The ammonium citrate and sodium acetate inhibit the growth of many organisms, including streptococci, molds and members of the oral microbial flora other than lactobacilli, and restrict swarming on the agar medium.<sup>9</sup> The low pH, due to the addition of acetic acid, also is inhibitory for other bacterial flora and favors the isolation of lactobacilli.

## Formulae

### BBL™ LBS Agar

Approximate Formula* Per Liter		
Pancreatic Digest of Casein .....	10.0	g
Yeast Extract .....	5.0	g
Monopotassium Phosphate .....	6.0	g
Ammonium Citrate .....	2.0	g
Dextrose .....	20.0	g
Polysorbate 80 .....	1.0	g
Sodium Acetate Hydrate .....	25.0	g
Magnesium Sulfate .....	575.0	mg
Manganese Sulfate .....	0.12	g
Ferrous Sulfate .....	34.0	mg
Agar .....	15.0	g

### BBL™ LBS Broth

Consists of the same ingredients without the agar.

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

## Directions for Preparation from Dehydrated Product

1. Suspend/dissolve the powder in 1 L of purified water:  
**BBL™ LBS Agar** – 84 g (for growth of *L. acidophilus*, add 84 g to 200 mL of filtered tomato juice plus 800 mL of purified water);  
**BBL™ LBS Broth** – 70 g.  
Mix thoroughly.
2. Add 1.32 mL of glacial acetic acid (ACS).
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
4. Cool and use without autoclaving. If storage is necessary, autoclave at 118°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

## User Quality Control

### Identity Specifications

#### BBL™ LBS Agar

Dehydrated Appearance: Fine, slightly clumped and moist, homogeneous, free of extraneous material.

Solution: 8.4% solution, soluble in purified water upon boiling. Solution (with acetic acid) is light to medium, yellow to tan, moderately hazy to hazy.

Prepared Appearance: Light to medium, yellow to tan, moderately hazy.

Reaction of 8.4% Solution at 25°C: pH 5.5 ± 0.2

#### BBL™ LBS Broth

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 7.0% solution, soluble in purified water upon boiling. Solution (with acetic acid) is light to medium, yellow to tan, slightly hazy to clear.

Prepared Appearance: Light to medium, yellow to tan, slightly hazy to clear.

Reaction of 7.0% Solution at 25°C: pH 5.4 ± 0.2

### Cultural Response

#### BBL™ LBS Agar or LBS Broth

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C with 3-5% CO<sub>2</sub> for 42-48 hours (up to 4 days, if necessary).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Lactobacillus acidophilus</i>	4356	Undiluted	Good
<i>Lactobacillus plantarum</i>	8014	Undiluted	Good
<i>Proteus vulgaris</i>	8427	10 <sup>4</sup> -10 <sup>5</sup>	Partial to complete inhibition

## Procedure

LBS Agar can be used as a plating medium for counting lactobacilli in various types of specimens. Alternatively, it can be used for direct recovery of organisms using the streak-inoculation technique. Incubate plates at  $35 \pm 2^\circ\text{C}$  in an aerobic or anaerobic atmosphere supplemented with carbon dioxide.

Inoculate the broth with the test specimen and incubate tubes with loosened caps at  $35 \pm 2^\circ\text{C}$  in an aerobic or anaerobic atmosphere supplemented with carbon dioxide.

## Expected Results

On the agar medium, lactobacilli appear as large, white colonies. Growth should be subcultured to appropriate agar or broth media for use in biochemical identification procedures.

After growth has been obtained in LBS Broth, as evidenced by the appearance of turbidity in the tube, subculture aliquots to appropriate plating media (e.g., LBS Agar) in order to obtain isolates in pure culture for subsequent use in identification procedures.

## Limitations of the Procedure

1. LBS Agar should not be used for the maintenance of lactobacilli, since it is not sterile and, being a highly selective medium, is not favorable for keeping cultures in their normal state. Colonies required for further study should be subcultured as soon as possible.
2. This medium is not suitable for the isolation of dairy lactobacilli such as *L. lactis* and *L. bulgaricus* due to its content of salts.<sup>9</sup>

## References

1. Rogosa, Mitchell and Wiseman. 1951. J. Bacteriol. 62:132.
2. Rogosa, Mitchell and Wiseman. 1951. J. Dental Res. 30:682.
3. Wiseman, Sarles, Benton, Harper and Elvehjem. 1956. J. Bacteriol. 79:723.
4. Ellis and Sarles. 1958. J. Bacteriol. 75:272.
5. Rogosa and Sharpe. 1960. J. Gen. Microbiol. 23:197.
6. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
7. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
8. Sabine and Vaselekos. 1965. Nature 206:960.
9. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

## Availability

### BBL™ LBS Agar

COMPF SMD

Cat. No. 211327 Dehydrated – 500 g\*

### BBL™ LBS Broth

Cat. No. 211331 Dehydrated – 500 g\*

\*Store at 2-8°C.