Litmus Milk

Intended Use

Litmus Milk is used for the maintenance of lactic acid bacteria and as a differential medium for determining the action of bacteria on milk.

Summary and Explanation

Litmus Milk has been used for many years for determining the metabolic activities of microorganisms in milk as an aid to the identification of bacterial species. It is especially useful in species differentiation within the genus *Clostridium*.

This medium is also of value in the maintenance and propagation of lactic bacteria.

User Quality Control

Identity Specifications BBL[™] Litmus Milk

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	10.0% solution, soluble in purified water. Solution is medium, purple gray, opaque.
Prepared Appearance:	Medium, purple gray, opaque.
Reaction of 10.0% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response BBL[™] Litmus Milk

Prepare the medium per label directions. Inoculate with fresh cultures diluted 1:10 and incubate at 35 \pm 2°C for 7 days.

ORGANISM	ATCC™	RESULT
Clostridium perfringens	13124	Stormy fermentation (gas), clot or curd, reduction (white)
Lactobacillus acidophilus	4356	Acid (pink), clot or curd

Principles of the Procedure

Skim milk is the substrate that particular species of bacteria attack in different ways to produce various metabolic products. Azolitmin serves as a pH indicator with a color range of pink (below pH 4.5) to purple (in middle of pH range) to blue (above pH 8.3) and also functions as an Eh (oxidation-reduction) indicator.¹

The action of bacteria on milk can be categorized as follows:

- 1. No change (no carbohydrate fermentation and no change of litmus indicator).
- 2. Fermentation of lactose and/or dextrose in the milk with production of acid (pink color), including stormy fermentation (strong evolution of gas) by certain strains of *Clostridium*.
- 3. Action of proteolytic enzymes on lactalbumin with production of ammonia or basic amines resulting in an alkaline reaction (blue color).
- 4. Coagulation of casein as evidenced by the formation of a curd or clot. If the casein is converted to paracasein by the enzyme rennin, a clear, watery liquid called "whey" is produced at the top of a thoroughly coagulated tube.¹

- 5. Peptonization due to digestion of the milk protein as evidenced by a clearing of the medium and dissolution of the clot.
- 6. Reduction of the litmus in the depths of the tube due to the action of reductase enzymes with the resultant removal of oxygen to form the decolorized leucolitmus compound.

Formula

BBL[™] Litmus Milk

Approximate Formula* Per Liter	
Skim Milk	g
Azolitmin0.5	g
Sodium Sulfite0.5	g
*Adjusted and/or supplemented as required to meet performance criteria.	



Directions for Preparation from Dehydrated Product

- 1. Dissolve 100 g of the powder in 1 L of purified water, preferably preheated to 50°C. Mix thoroughly.
- 2. Autoclave at 115°C for 20 minutes or by fractional steam sterilization for 30 minutes on three successive days. Avoid overheating and the consequent caramelization which occurs.
- 3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes of Litmus Milk with 18- to 24-hour pure cultures. For the study of anaerobic organisms, sterile mineral oil can be layered over the medium following inoculation. Incubate tubes at $35 \pm 2^{\circ}$ C for up to 14 days and record reactions at various intervals during the incubation process.



Expected Results

Consult an appropriate reference for the expected reactions for specific microbial species.²

References

- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
 Sneath and Holt (ed.). 1986. Bergey's Manual[™] of determinative bacteriology, vol. 2. Williams & Wilkins, Baltimore, Md.

Availability

BBL[™] Litmus Milk

Cat. No. 211343 Dehydrated – 500 g

221657 Prepared Tubes – Pkg. of 10*

*Store at 2-8°C.

