

## LITMUS MILK

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

TYPICAL FORMULA	(g/ l)
Skim milk	100.0
Litmus	0.75
Final pH = 6.8 ± 0.2 at 25 °C.	

### DESCRIPTION

Litmus milk is used 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.

### PRINCIPLE

Skim milk is the substrate that particular species of bacteria attack in different ways to produce various metabolic products. The addition of a litmus indicator to milk expands its usefulness as a differential medium. Litmus incorporated in milk is both a pH indicator and an oxidation- reduction indicator.

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

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.
5. Peptonization due to the 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 leucolitus compound.

### PREPARATION

Dissolve 100.8 g of powder in 1 litre distilled or deionized water.

Sterilize in autoclave at 115 °C for 5 minutes.

Dispense into final containers.

### TECHNIQUE

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 ± 2°C for up to 14 days and record reactions at various intervals during the incubation process.

### INTERPRETATION OF RESULTS

If lactose is fermented, the solution turns pink. If gas is produced during fermentation, bubbles or cracks are visible in the milky medium.

If lactose is not fermented and proteins are used for energy, the solution will become alkaline and blue.

### PERFORMANCE AND LIMITATIONS

Avoid overheating during sterilization, as the milk sugar will caramelize, resulting in discoloration giving an appearance atypical of the sterile medium.

During the sterilization period, Litmus Milk is reduced to a white colored base, however upon cooling, the original color returns as oxygen is absorbed.

Reactions observed in Litmus Milk are not sufficient to speciate. Additional biochemical tests must be performed.

### STORAGE

The powder is very hygroscopic: store the powder at 10-30 °C, in a dry environment, in its original container tightly closed and use it before the expiry date on the label or until signs of deterioration or contamination are evident.

Store prepared media at 2-8°C.

### WARNING and PRECAUTIONS

The product is not classified as hazardous by current legislation and does not contain harmful substances in concentrations of ≥1%.

The product is designed for *In vitro* diagnostic use and must be used only by properly trained operators.

### DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

### REFERENCES

1, MacFaddin, J.D. (1985). Media for isolation- cultivation- identification- maintenance medical bacteria, vol 1, p. 275-284, Williams & Wilkins, Baltimore, MD.



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## PRODUCTION SPECIFICATIONS

### NAME

LITMUS MILK.

### PRESENTATION

Dehydrated culture medium.

### STORAGE

10-30°C.

### PACKAGING

Code	Content	Packaging
610318	500 g	500 g of powder in plastic bottle
620318	100 g	100 g of powder in plastic bottle

### pH OF THE MEDIUM

6.8 ± 0.2

### USE

Litmus milk is used 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.

### TECHNIQUE

Refer to technical sheet of the product.

### APPEARANCE of the MEDIUM

Dehydrated medium

Appearance: free-flowing, homogeneous.

Color: grayish-purple.

Prepared medium

Appearance: opaque.

Color: purple- gray.

### SHELF LIFE

4 years.

### QUALITY CONTROL

1. Control of general characteristics, label and print

2. Sterility control

7 days at 25 ± 1°C, in aerobiosis

7 days at 36 ± 1°C, in aerobiosis

3. Microbiological control









Inoculum for productivity: 10-100 UFC/ml

Inoculum for specificity: ≤ 10<sup>4</sup> UFC/ml

Incubation conditions: 35 ± 2 °C for 7 days.

Microorganism	ATCC	Result
<i>Clostridium perfringens</i>	13124	Stormy fermentation (gas), clot or curd, reduction (white)
<i>Lactobacillus acidophilus</i>	4356	Acid pink, clot or curd

### TABLE OF SYMBOLS

 Batch code	 Temperature limitation	 Manufacturer	 Contains sufficient for <n> tests
 Catalogue number	 Keep away from heat	 Use by	 Caution, consult accompanying documents



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