

# PRODUCT SPECIFICATION SHEET

## Litmus Milk (DM134)

### Intended Use

Litmus Milk (DM134) is recommended for maintenance of lactic acid bacteria and for determining the action of bacteria on milk.

### Product Summary and Explanation

For several years Litmus Milk has been used for determining the metabolic activities of microorganisms in milk as an aid to the identification of bacterial species. Milk contains the carbohydrate lactose along with three main proteins i.e. casein, lactalbumin and lactoglobulin.<sup>(1)</sup> Therefore, an organism may demonstrate one or several of the following metabolic properties in litmus milk, each specific for a particular species aiding bacterial identification. The various metabolic functions are lactose fermentation, litmus reduction, clot formation, peptonization (digestion) and gas formation.<sup>(2)</sup> 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. Litmus Milk is the most useful medium in dairy industry as it is a reliable indicator of bacterial action on milk.<sup>(3)</sup> Litmus is a good indicator of acidity, alkalinity and its oxidation-reduction potential and as compared to bromocresol purple it is useful in milk media with lower toxicity to microorganisms.<sup>(4)</sup> Litmus Milk accelerates the growth of some organisms, which cannot grow in plain Litmus Milk by addition of 1% w/v dextrose and/or 5% w/v yeast extract.<sup>(3, 4, 5)</sup>

### Principles of the Procedure

Litmus milk contains skim milk which is a substrate that particular species of bacteria attack in different ways to produce various metabolic products. Litmus 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.

### Formula / Liter

Ingredients	Gms / Liter
Skim milk powder	100.00
Litmus	0.50
Sodium sulphite	0.50
Final pH: 6.8 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 101 grams of the medium in one liter of distilled water, agitating continuously.
2. Dispense 10 ml amounts into 15 x 150 mm tubes.
3. Autoclave at 121°C, 15 psi pressure, for 5 minutes / validated cycle.
4. AVOID OVERHEATING

### Quality Control Specifications

Dehydrated Appearance	Pinkish purple to grey homogeneous free flowing powder may contain minute to small particles
Prepared Medium	Light purple coloured opaque milky solution
Reaction of 10.1% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Not Applicable

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for upto 14 days and record the reactions of various intervals during the incubation.

Sr. No.	Organisms	Results to be achieved	
		Inoculum	Growth

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		(CFU)	
1.	<i>Clostridium perfringens</i> ATCC 13124	good-luxuriant	stormy fermentation (gas)
2.	<i>Lactobacillus acidophilus</i> ATCC 11506	good-luxuriant	acid clot (pink)
3.	<i>Pseudomonas aeruginosa</i> ATCC 27853	good-luxuriant	peptonisation (clearing)

The organisms listed are the minimum that should be used for quality control testing.

## Test Procedure

- For detection of *Clostridium perfringens* in water, inoculate freshly heated tubes of Litmus Milk with various quantities of water and heat at 80°C for 10-15 minutes to destroy non-spore-forming organisms.
- Examine after every 24 hours for positive Stormy Clot reaction at 35°C for up to 5 days.
- Anaerobiosis in Litmus Milk can be obtained by adding a small heated iron nail or 0.1 gram of reduced iron to the medium. Sterile mineral oil can be layered over the medium following inoculation.

## Results

Skim milk is the substrate, metabolized by particular species of bacteria in different ways. The actions of bacteria can be categorized as follows:

### a) ACID REACTION

- Pink to red colour Fermentation of lactose of the milk and/or dextrose in milk.
- Acid coagulation Lactic acid production, producing a casein curd in clear watery fluid.
- Stormy clot Gas formation in coagulated casein curd.

### b) ALKALINE REACTION

- Blue colour of the Formation of basic amines or ammonia milk due to proteolysis.
- Alkaline coagulation Paracasein formation from casein by enzyme rennin with a soft, blue clot.
- Peptonization Digestion of casein, evident by clearing of the medium and dissolution of the clot.

### c) REDOX REACTION

- Decolourized medium Reaction of Litmus in the depths of (Similar to freshly the tube by reductase enzymes with autoclaved Litmus the resultant removal of oxygen to Milk) form the decolourized leucolimus compound.
- Reactions obtained in this medium are not specific and further tests must be carried out.

Refer appropriate references and procedures for interpretation of results.

## Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

## Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

## Limitations of the Procedure

- For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
- Consult appropriate texts for detailed information and recommended procedures.

## Packaging

Product Name : Litmus Milk

Product Code : DM134

Available Pack sizes : 100gm/500gm

## References

- Cantarow A., Schepartz B., Biochemistry, 3rd Ed., Philadelphia: W B Saunders, 1962:273,792-793
- MacFaddin J. F., 2000, Biochemical tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.

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3. Davis J. G., 1935, J. Dairy Res., 6:121.
4. Davis J. G., 1955, A Dictionary of Dairying, 2nd Ed., Leonard Hill.
5. Davis J. G., 1959, Milk Testing, 2nd Ed., United Trade Press.

### Further Information

For further information please contact your local MICROMASTER Representative.



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