

# LITMUS MILK MEDIUM

**PRODUCT:****Tube Media:**

Litmus Milk, item no. T6790

**PURPOSE:**

Litmus Milk is a differential medium with a variety of uses. Organisms can be differentiated on the basis of multiple metabolic reactions in this medium. It is used to aid in the speciation of *Clostridium*, the differentiation of *Streptococcus bovis* from *Streptococcus equinus*, the maintenance of lactic acid bacteria in dairy products, the identification of *Bacillus anthracis*, and is used for the detection of *Bacillus cereus* in foods. It is also an aid in the presumptive identification of *Propionibacterium* species.

**PRINCIPLE:**

Milk was one of the first substrates used to propagate and maintain bacteria. The medium is made differential by the addition of litmus, which serves as a pH indicator as well as an oxidation-reduction indicator. Milk contains the carbohydrate lactose and the three proteins casein, lactalbumin, and lactoglobulin. Organisms may exhibit one or several metabolic properties, such as lactose fermentation, reduction of litmus, clot formation, peptonization (digestion), or gas formation.

**FORMULA:**

Approximate, per liter deionized filtered water.

Skim Milk .....	100.0 g
Litmus .....	750.0 mg
Final pH 6.8 ± 0.2 at 25°C	

**PRECAUTIONS:\***

For in vitro diagnostic use. Observe approved biohazard precautions.

**Storage:** Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (evaporation or discoloration), or if the expiration date has passed.

**Limitations:** A clot formation is simply recorded as "clot". Do not attempt to differentiate between a clot and curd formation in this medium.

Upon storage the tubes may have a layered coloration. This is due to the oxidation of the top portion of the tube and should not affect the results of the test.

Reactions observed in Litmus Milk are not sufficient to speciate; additional biochemical and serological tests must be performed for definitive identification.

**PROCEDURE:\***

**Specimen Collection:** Information on specimen collection and transport is found in standard reference material on the subject. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory without delay.

**Method of Use:** Prior to inoculation, the medium should be brought to room temperature. Inoculate approximately 4 drops from an 18- to 24-hour pure broth culture, or a single isolated colony from TSI or other suitable medium. If *Clostridium* is suspected or anaerobiosis is desired, incubate anaerobically, or alternately, add sterile reduced iron (iron powder, nails, paper clips, or metal filings) to the tube, or pour a layer of sterile mineral oil or Vaspar over the medium surface immediately after inoculation and incubate aerobically at 35°C for 18-24 hours. Longer periods of incubation may be necessary; the medium may be held up to 14 days. Observe daily for metabolic reactions including acid production, alkalinity, indicator reduction, clot formation, peptonization, or gas production.

Interpretation: The following reactions may be observed:

1. **Acid Reactions:**  
 Pink: Litmus has changed color due to the fermentation of glucose.  
 Red: Litmus has changed color due to the fermentation of lactose.  
 Clear watery fluid expressed from curd: Acid coagulation due to the lactic acid uniting with calcium to form calcium lactate, resulting in the precipitation of casein.
2. **Alkaline Reactions:**  
 Blue: Litmus has changed color due to the breakdown of lactalbumin, due to proteolytic enzymes, resulting in the formation of ammonia and basic amines.  
 Alkaline coagulation: Caused by the conversion of casein to paracasein by the enzyme rennin.  
 Clearing of medium: Caused by peptonization due to the digestion of casein. The resulting fluid is more viscous and turbid than whey.
3. **Reduction:**  
 White medium: Reduction is due to the enzyme reductase which removes the oxygen from the litmus and leaves decolorized leucolitus. Reduction usually begins at the bottom of the tube where aeration is reduced.
4. **Clot or curd formation:**  
 Soft, gelatinous clot which may or may not retract from the sides of the tube, due to milk protein coagulation.
5. **Gas production:**  
 Bubbles occur in the medium. Stormy fermentation results when there is an abundance of gas resulting in the breakup of an acid clot. This reaction is characteristic of *Clostridium* species.
6. **No change in the medium:**  
 Reaction is negative.

**Materials Required but Not Provided:** Standard microbiological supplies and equipment such as loops, needles, pipettes, incubator, and incinerator are not provided.

**QUALITY CONTROL:\***

<b>Microorganisms Used (ATCC #):</b>	<b>Expected Results:</b>
<i>Clostridium perfringens</i> (13124)	Glucose fermentation; clot formation; gas production
<i>Moraxella osloensis</i> (10973)	No change
<i>Pseudomonas aeruginosa</i> (27853)	Alkaline reaction at surface Key: See "Interpretation"

**User Quality Control:** Check for signs of contamination and deterioration.

**BIBLIOGRAPHY:**

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5. Webster, G. F., and K. J. McGinley, *J. Clin. Microbiol.*, 5:661, 1977.

\*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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