



## Milk Medium with a reducing agent

M816

Milk medium w/ reducing agent is used for determination of litmus reaction of *Clostridium* species.

### Composition\*\*

Ingredients	Gms / Litre
Skim milk	100.000
Peptic digest of animal tissue	10.000
Sodium thioglycollate	0.500
Litmus	5.000
Final pH ( at 25°C)	7.0±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 115.5 grams in 1000 ml distilled water agitating continuously. Sterilize by autoclaving at 15 lbs pressure (121°C) for 5 min. Mix well and dispense as desired.

### Principle And Interpretation

Milk is a complex nutritional source that contains proteins (mainly casein) in an aqueous solution of lactose and minerals. Bacterial enzymes alter the media and may bring about various changes. Litmus is added to the medium to detect pH changes that may occur as a result of these enzymatic reactions. Above pH 8.3, litmus is blue, while below pH 4.5 litmus is red. Fermentation of lactose results in the production of acid, which causes milk to curdle or form a clot at the bottom of the tube. Litmus may also act as an electron acceptor thus becoming reduced by bacterial metabolism. This reaction is observed as a white color in the medium. Milk medium with reducing agent is used for determination of litmus reaction of *Clostridium* species.

Peptic digest of animal tissue and skim milk provide nitrogen, sulphur, vitamins and other growth nutrients. Sodium thioglycollate is a reducing agent, which absorbs oxygen and creates a reduced environment required by anaerobes. This medium has been found satisfactory for the cultivation of *Clostridium* species and allows observation of their reactions in litmus milk. In anaerobically grown Litmus Milk cultures, enzymes of *Clostridium perfringens* attack the proteins and carbohydrates of the milk producing a stormy fermentation with clotting and gas formation (1).

### Quality Control

#### Appearance

Light pink to purple homogeneous free flowing powder

#### Colour and Clarity of prepared medium

Purple coloured opalescent solution

#### Reaction

Reaction of 11.55% w/v aqueous solution at 25°C. pH : 7.0±0.2

#### pH

6.80-7.20

#### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for upto 5 days.

#### Cultural Response

Organism	Observation	Cause
<b>Cultural Response</b> <i>Clostridium perfringens</i> ATCC 12924	stormy fermentation	gas trapped in acid coagulated casein peptonization

*Clostridium sporogenes* acid with gas -  
*ATCC11437* proteolysis

### Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

### Reference

1. Gainor C. and Wegemer D. E., Appl. Microbiol., 1954 March; 2(2): 9597.

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