Neutral Red Chalk Lactose Agar

M984

Intended Use:

Recommended for detection of lactic Streptococci in milk and milk products.

Composition**

Ingredients	Gms / Litre
Peptone	3.000
HM peptone B #	3.000
Yeast extract	3.000
Lactose	10.000
Calcium carbonate	15.000
Neutral red	0.050
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 49.05 grams in 1000 ml purified/distilled water. Heat just to boiling. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates with intermittent shaking. *Note: Due to presence of Calcium Carbonate, the prepared medium forms opalescent solution with white precipitate.*

Principle And Interpretation

Lactic Streptococci are normally present in milk and are also used as starter cultures in the production of cultured dairy products (5). The natural microflora of milk is inefficient, uncontrollable, and unpredictable, or is destroyed altogether by the heat treatments given to the milk. A starter culture can provide particular characteristics in a more controlled and predictable fermentation. The primary function of lactic starters is the production of lactic acid from lactose.
Peptone, HM peptone B and yeast extract provide a source of nitrogen and other growth factors. Lactose is the fermentable carbohydrate. Neutral red is the pH indicator used in this medium. As it is unable to prevent diffusion of acidic or basic byproducts throughout the agar, resulting in an overall color change of the entire medium toward the acidic or basic range, calcium carbonate is often added which acts as a non-diffusible buffer. Thus the acid produced by any colony is localized around it (4).

Type of specimen

Dairy samples - Milk and Milk products.

Specimen Collection and Handling:

For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Due to nutritional variations of organisms certain strains may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared medium
Pink coloured opalescent gel with white precipitate forms in Petri plates

Reaction
Reaction of 4.91% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Streptococcus thermophilus ATCC 14485	50-100	luxuriant	>=50%

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Reddy M. S., Vedamuthu E. R., Washam C. J. and Reinbold G. W., 1969 Appl. Microbiol., 18, 755.
5. Seppo Salminen, Atte von Wright and Arthur Ouwehand, Lactic Acid Bacteria. Microbiological and Functional aspects, 3rd Edition, Marcel and Dekker, NY. Basel.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

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