HiCrome™ Lactobacillus Selective Agar Base

M2065

Intended Use:

Recommended for selective isolation and differentiation of *Lactobacillus* from mixed culture by chromogenic method.

Composition**

Ingredients	g / L
Peptone	10.000
HM Extract #	1.000
M-Protein powder ##	5.000
D-Mannitol	10.000
Sodium chloride	10.000
Chromogenic mixture	3.200
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat Extract

Equivalent to Milk Protein

Directions

Suspend 54.22 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121ºC) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Cip selective supplement (FD345). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacillus is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria. They are a major part of the lactic acid bacteria group. As more LABs have been developed and sold in mixed forms as probiotics, it is necessary to develop a method for counting each LAB in a mixture (1).

The medium contains peptone and HM extract, which provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by phenol red. M-protein aids in detecting casein hydrolysis activity. The chromogenic mixture present in the medium is cleaved by the enzyme beta-glucosidase resulting in greenish blue to blue coloured colonies. For selective isolation of Lactobacillus, Cip selective supplement (FD345)is added which inhibits the accompanying bacteria.

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 (2,3).

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
4. Further biochemical and serological test are need to be carried out for confirmation.

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 pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.42% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 24-48 hours with addition of Cip selective supplement (FD345)(with 5% CO_2).

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Lactobacillus acidophilus ATCC 4356 (00098*)	50-100	good-luxuriant	>=50%	Pale pink - pink
$*Lactobacillus paracasei* ATCC 9595	50-100	good-luxuriant	>=50%	Light green
Lactobacillus fermentum ATCC 9338	50-100	good-luxuriant	>=50%	Yellow
Lactobacillus plantarum ATCC 8014	50-100	good-luxuriant	>=50%	Light green-green colonies w/ hazy background
Lactococcus lactis subsp. *lactis* ATCC 19435 (00016*)	50-100	good-luxuriant	>=50%	Light green-green colonies w/ hazy background
**Bacillus spizizenii* ATCC 6633 (00003*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Bacillus cereus ATCC 10876	>=10^4	inhibition	0%	

Key : (*) Corresponding WDCM numbers and $ Formerly known as *Lactobacillus casei,* **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°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 (4,5).

Reference

1.De Man, J.C., Rogosa, M. and Sharpe, E.M. (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23, 30–35.

2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.

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