

For isolation and differentiation between various species of *Lactobacillus*

HiCrome™ Lactobacillus Selective Agar Base

Recommended for isolation and differentiation between various species of *Lactobacillus* from a mixed culture by chromogenic method

M2065

Composition **

Ingredients	Grams/Litre
Peptone	10.00
HM Extract #	1.00
M-Protein powder ##	5.00
D-Mannitol	10.00
Sodium chloride	10.00
Chromogenic mixture	3.20
Phenol red	0.025
Agar	15.00

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 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Ciprofloxacin 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 β -glucosidase resulting in greenish blue to blue coloured colonies.

For selective isolation of *Lactobacillus*, Ciprofloxacin supplement is added (FD345) 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, processing as per guidelines and local standards (2, 3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

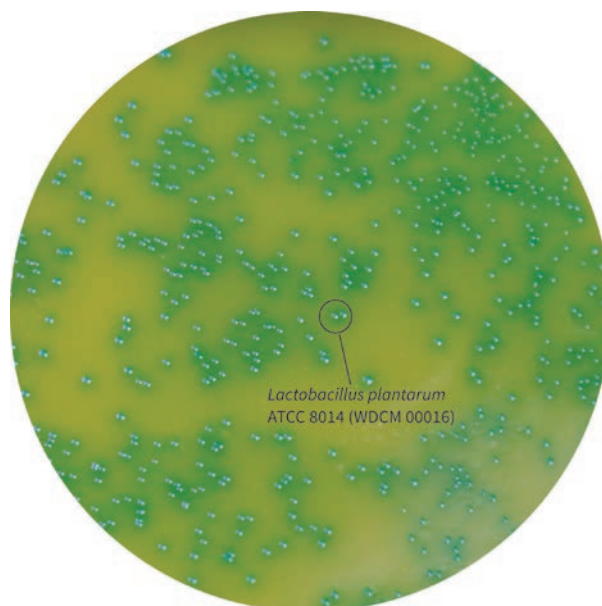
Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety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

Limitations

1. Some species may show poor growth due to nutritional variations.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

Performance and Evaluation

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



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Quality Control

- Appearance of Powder** : Light yellow to pink homogeneous free flowing powder.
- Gelling** : Firm, comparable with 1.5% Agar gel
- Colour and Clarity** : 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
- Cultural Response** : Cultural characteristics observed with addition of Ciprofloxacin supplement (FD345) after an incubation at 25-30°C for 24-48 hours (with 5% CO₂).

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Lactobacillus acidophilus</i> ATCC 4356 (WDCM 00098)	50-100	good - luxuriant	>=50%	Pale pink - pink
<i>Lactobacillus rhamnosus</i> ATCC 9595	50-100	good	>=50%	Light green
<i>Lactobacillus fermentum</i> ATCC 9338	50-100	good - luxuriant	>=50%	Yellow
<i>Lactobacillus plantarum</i> ATCC 8014 (WDCM 00016)	50-100	good - luxuriant	>=50%	Light green-green colonies w/ hazy back-ground
<i>Lactococcus lactis</i> subsp. <i>lactis</i> ATCC 19435	50-100	good - luxuriant	>=50%	Light green-green colonies w/ hazy back-ground
<i>Bacillus spizizenii</i> subsp. <i>spizizenii</i> ATCC 6633 (WDCM 00003)	>=10 ³	inhibited	0%	
<i>Bacillus cereus</i> ATCC 10876	>=10 ³	inhibited	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (WDCM 00032)	>=10 ³	inhibited	0%	

Storage and Shelf-life

Store between 2-8°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. Use before expiry date on the label.

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).

References

- De Man, J.C., Rogosa, M. and Sharpe, E.M. (1960) A medium for the cultivation of lactobacilli. J Appl Bacteriol 23, 30-35.
- American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington, D.C.
- Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
- Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
- 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.