



HiCrome™ Nickels and Leesment Medium

M1712

Intended Use

Recommended for the enumeration of citrate-fermenting lactic acid bacteria from milk, milk products and mesophilic starter cultures.

Composition**

Ingredients	g / L
Tryptone	18.000
Yeast extract	4.500
Gelatine	2.250
Glucose (Dextrose)	4.500
Lactose	4.500
Sodium chloride	3.600
Trisodium citrate dihydrate	1.800
Calcium lactate pentahydrate	8.000
Tricalcium dicitrate tetrahydrate	6.650
Carboxymethyl cellulose (CMC)	0.400
Chromogenic substrate (X-gal)	0.200
Agar	15.000
Final pH (at 25°C)	6.65±0.05

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 66.0 gram (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/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. If desired, add rehydrated contents of 2 vials of Van100 Selective supplement (FD245). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactic acid bacteria are widespread in nature and are best known for their activities in major food such as dairy, meat and vegetable products (1).

Testing for lactic acid bacteria in dairy products may be useful for various reasons like evaluating lactic starter cultures; determining the cause of acid defects in milk products, controlling the quality of cured cheese, cultured milks and uncultured products containing added cultures (2).

HiCrome™ Nickels and Leesment Medium is a modification of Modified Nickels and Leesment Medium formulated as per APHA (3) and is used for the enumeration of citrate-fermenting lactic acid bacteria using colony count technique at 25°C. Tryptone and yeast extract serve as carbon and nitrogen sources, long chain amino acids, vitamins and other essential growth nutrients. Lactose and glucose are the carbohydrate source in the medium. X-gal differentiates between *Lactococcus lactis* subsp. *lactis* and *Leuconostoc* species. *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* colonies are white with a clear zone. *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* colonies are white without a clear zone. *Leuconostoc* species are blue, with or without a clear zone. HiCrome™ Nickels and Leesment Medium with the addition of Van100 Selective supplement (FD245) can be used for the selective isolation of *Leuconostoc* from a mix flora of lactic acid bacteria. Sodium chloride maintains osmotic equilibrium and various salts provides essential ions.

Type of specimen

Food and dairy samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3,4). 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 variable nutritional requirements, some strains show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.
3. Further biochemical and serological test needs 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

Cream to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

White coloured, opaque gel containing white precipitate forms in Petri plates.

Reaction

Reaction of 6.6% w/v aqueous solution at 25°C. pH : 6.65±0.05

pH

6.60 - 6.70

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 48-72 hours.

Organism	Growth	Growth with FD245	Colour of colony
<i>L. lactis</i> biovar <i>diacetylactis</i>	good-luxuriant	inhibited	white with a clear zone
<i>L. lactis</i> subsp <i>lactis</i> ATCC 19435 (00016*)	good-luxuriant	inhibited	white without a clear zone
<i>L. lactis</i> subsp <i>cremoris</i> ATCC 19257	good-luxuriant	inhibited	white without a clear zone
<i>Leuconostoc mesenteroides</i> ATCC 9135 (00108*)	good-luxuriant	good-luxuriant	blue without clear zone

Key : (*) Corresponding WDCM numbers.

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 (5,6).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Marshall R.T., 1992, Standard Methods for the Examination of Dairy products, 16th Ed, American Public Health Association, Washington D.C.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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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