HiCrome™ Nickels and Leesment Medium

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

M1712

Composition **

Ingredients	Grams/Litre
Tryptone	18.00
Yeast extract	4.50
Gelatine	2.25
Glucose (Dextrose)	4.50
Lactose	4.50
Sodium chloride	3.60
Trisodium citrate dihydrate	1.80
Calcium lactate pentahydrate	8.00
Tricalcium dicitrate tetrahydrate	6.65
Carboxymethyl cellulose (CMC)	0.40
Chromogenic substrate (X-gal)	0.20
Agar	15.00

Final pH (at 25°C) 6.65 ± 0.05

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 66.0 grams (the equivalent weight of dehydrated medium per litre) 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. If desired, add rehydrated contents of 2 vials of HiCrome™ Nickels and Leesment 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 (1) 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, vitamin B complex and other essential growth nutrients. Lactose and Glucose (Dextrose) 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 HiCrome™ Nickels and Leesment Supplement (FD245) can be used for enumeration of *Leuconostoc* (1). Vancomycin acts as a supplement for the selective isolation of *Leuconostoc* from a mix flora of lactic acid bacteria. Sodium chloride maintains osmotic equilibrium and various salts provide essential ions.

Type of specimen

Dairy : milk and milk product samples

Specimen Collection and Handling

For dairy samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (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.

M1712 HiCrome™ Nickels and Leesment Medium

HiCrome™ VegFreedom from BSE / TSE worries

Single Streak Rapid Differentiation Series

HiCrome™ Nickels and Leesment Medium (M1712) is also available as HiCrome™ Nickels and Leesment HiVeg™ Medium (MV1712) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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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 may show poor growth on this medium.
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.

Quality Control

Appearance of Powder : Cream to light yellow coloured homogeneous, free flowing powder.
Gelling : Firm, comparable with 1.5% Agar gel.
Colour and Clarity of prepared medium : White coloured opaque gel forms in Petri plates containing white precipitate.
Reaction : Reaction of 6.6% w/v aqueous solution at 25°C. pH : 6.65 ± 0.05.
Cultural Response : Cultural characteristics observed after an incubation at 25-30°C for 48-72 hours.

Organisms (ATCC)	Growth	Growth**	Colour of colony
Lactococcus lactis subsp *lactis biovar diacetylactis*	good-luxuriant	inhibited	white with a clear zone
Lactococcus lactis subsp lactis (19435) (00016*)	good-luxuriant	inhibited	white without a clear zone
Lactococcus lactis subsp *cremoris* (19257)	good-luxuriant	inhibited	white without a clear zone
Leuconostoc mesenteroides (9135) (00108*)	good-luxuriant	good-luxuriant	blue without a clear zone

Key : * : Corresponds to WDCM number
** = with the addition of HiCrome Nickels and Leesment Selective Supplement (FD245)

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 inorder 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

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2. Marshall R.T., 1992, Standard Methods for the Examination of Dairy products, 16th Ed, American Public Health Association, Washington D.C.
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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.
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