

Specification

Culture medium for lactobacilli supplemented with cystine.

Presentation

20 Prepared Plates
 90 mm
 with: 21 ± 2 ml

Packaging Details

1 box with 2 packs of 10 plates/pack. Single cellophane.

Shelf Life

3,5 months

Storage

2-14 °C

Composition

Composition (g/l):

| | |
|----------------------------|------|
| Proteosa Peptone..... | 10.0 |
| Meat extract..... | 8.0 |
| Yeast extract..... | 4.0 |
| D-(+)-Glucose..... | 20.0 |
| Sodium acetate..... | 5.0 |
| Magnesium sulfate..... | 0.2 |
| Manganese sulfate..... | 0.05 |
| Dipotassium phosphate..... | 2.0 |
| Triammonium citrate..... | 2.0 |
| Sorbitan mono-oleate..... | 1.0 |
| Cystein..... | 0.5 |
| Agar..... | 15.0 |

Description /Technique

Description:

MRS Agar is a medium used for the cultivation of lactobacilli. It is a modification of a medium based on the highly nutritious properties of tomato juice. The addition of magnesium, manganese and acetate, together with polysorbate, provides an improved medium for the growth of lactobacilli, including very fastidious species such as *Lactobacillus brevis* and *Lactobacillus fermentum*.

The quality of the peptones in addition to the meat and yeast extracts, combine all the necessary growth factors that make MRS medium one of the best media for the cultivation of lactobacilli.

As the selectivity of this medium is low and contaminants tend to grow subculturing in a (double layer) solid medium, and then in broth is recommended to increase selectivity. In many cases, growth is encouraged by incubation in a CO₂ enriched atmosphere.

MRS medium is particularly recommended for the enumeration and maintenance of lactobacilli either by the MPN technique in broth, or by inoculation on a plate, overlaying it with a second layer of molten medium. This technique overcomes the need for a CO₂ enriched atmosphere.

Note: the addition of cysteine enhances the growth of lactobacilli.

Technique:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results.

Spread the plate by streaking methodology or by spiral method. Incubate the plates right side up in a CO₂ atmosphere at 30 ±1°C for 72 ±3h.

(Incubation times longer than those mentioned above or different incubation temperatures may be required depending on the sample, on the specifications,...

This medium can be inoculated directly or after enrichment broth like MRS broth) Incubated under microaerophilic conditions to promote lactobacilli enrichment.

After incubation, enumerate all the colonies that have appeared onto the surface of the agar.

Each laboratory must evaluate the results according to their specifications.

Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by inverse dilution factor if streaked a diluted sample. Report results as Colony Forming Unit (CFU's) per ml or g along with incubation time and temperature.

Quality control

Physical/Chemical control

Color : Yellowish-brown pH: 5.9 ± 0.2 at 25°C

Microbiological control

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^3 - 10^4 (qualitative selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosi. Incubation at 30 ± 1 °C for 72 ± 3 h

Microbiological control according to ISO 11133:2014/A1:2018.

Microorganism

Escherichia coli ATCC® 25922, WDCM 00013

Lactococcus lactis ATCC® 19435, WDCM 00016

Pediococcus pentosaceus ATCC® 33316, WDCM 00158

Lactobacillus acidophilus ATCC® 4356, WDCM 00098

Lactobacillus fermentum ATCC® 9338

Growth

Poor to good

Good ($\geq 70\%$)

Good ($\geq 70\%$)

Good ($\geq 70\%$)

Good ($\geq 70\%$)

Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

Bibliography

- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington. DC. USA.
- FIL-IDF Standard 146 (2003) Yoghurt. Identification of characteristic microorganisms.
- IFU Method No. 5 (1996) Lactic Acid Bacteria Count Procedure. Schweizerischer Obstverband. CH-6302 Zug.
- IFU Method No. 7 (1998) Sterility testing of aseptic filled products, commercial sterile products and preserved products. Schweizerischer Obstverband. CH-6302 Zug.
- IFU Method No. 9 (1998) Microbiological examination of potential spoilage microorganisms of tomato products. Schweizerischer Obstverband. CH-6302 Zug.
- ISO Standard 9232 (2003) Yoghurt - Identification of characteristic microorganisms (*Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus*).
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO Standard 15214 (1998) Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony count technique at 30°C.
- MAN, J.C. de, ROGOSA, M. & SHARPE, M. Elisabeth (1960) A medium for the cultivation of lactobacilli. J. Appl. Bact.; 23:130.

Storage

Storage conditions: 2-14°C

Alternatively the plates may also be stored at the range of 2 - 25°C, with a proper performance of the medium, but some precautions must be taken into account:

-In the range of 2 - 8 °C avoid direct contact with surfaces that can freeze product.

-In the range of 15 - 25 °C, dehydration control must be taking in account.