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MRS agar

For microbiological control only

Enumeration of mesophilic lactic acid bacteria

SUMMARY AND EXPLANATION

The MRS agar (de Man, Rogosa and Sharpe) is a slightly selective medium which enables the growth of all species of lactic acid bacteria.

The formula of this medium complies with the International Standard NF ISO 15214 (2).

PRINCIPLE

The medium is used for the enumeration of mesophilic lactic acid bacteria by colony counting at 30°C.

It combines a rich peptone base with elements known to favor the development of lactic acid bacteria: sodium acetate, Tween® 80, ammonium citrate, manganese and magnesium salts.

Numerous descriptions of this medium can be found in the literature and in other older standards (NF V 04-503, ISO7889).

The medium's inhibitory capacity stems from its acidic pH. This is limited to certain enterobacteria strains and Gram-positive cocci.

According to the standard NF ISO 15214, if contamination by yeasts is suspected, sorbic acid can be added to the formula.

CONTENT OF THE KIT

| Ready-to-use medium | |
|-------------------------|----------------------------|
| | pH 5.4 |
| REF AEB521760A | Pack of 120 plates 90 mm |
| REF AEB621756A | Pack of 6 flasks of 100 ml |
| REF AEB621757A | Pack of 6 flasks of 200 ml |
| | pH 5.7 |
| REF AEB521761V | Pack of 120 plates 90 mm |
| REF AEB621756V | Pack of 6 flasks of 100 ml |
| REF AEB621757VAF | Pack of 6 flasks of 200 ml |
| | pH 6.4 |
| REF AEB621756N | Pack of 6 flasks of 100 ml |
| REF AEB621757N | Pack of 6 flasks of 200 ml |
| REF AEB521760 | Pack of 120 plates (90 mm) |

COMPOSITION

Theoretical formula.

This medium can be adjusted and/or supplemented according to the performance criteria required.

| | |
|--------------------------------------|--------|
| Casein peptone | 10 g |
| Meat extract | 10 g |
| Yeast extract | 4 g |
| Triammonium citrate | 2 g |
| Sodium acetate | 5 g |
| Magnesium sulfate heptahydrate | 0.2 g |
| Manganese sulfate tetrahydrate | 0.05 g |
| Dipotassium phosphate | 2 g |
| Glucose | 20 g |
| Tween® 80 | 1.08 g |
| Agar | 12 g |
| Purified water | 1 l |

MATERIAL REQUIRED BUT NOT PROVIDED

- Sterile Petri dishes.
- Water bath.
- Bacteriology incubator.

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29-A, *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Current Revision*". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest edition", or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not use reagents past the expiry date.
- Do not use bottles/plates which show signs of contamination.
- Before use, make sure the tamper-proof seal on the bottle stopper is intact.
- The medium should be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- **Store the bottles/plates in their box at 2-25°C until the expiry date.**

SPECIMENS

Follow the recommendations in the current standards to perform specimen collection and preparation.

INSTRUCTIONS FOR USE

Preparation:

1. Loosen the cap on the bottle of agar.
2. Place the bottle of agar in a water bath equipped with a security system set to approximately 50°C, increase the temperature to 100°C and leave the agar to melt (approximately 20-30 minutes).
3. Mix after screwing the cap back on (use protective gloves against thermal risks).
4. Leave the bottles at room temperature for at least 15 seconds before transferring them to a thermostatically controlled water bath set at 44-47°C.

Inoculation and incubation:

- Transfer 1ml of the stock solution or one of the dilutions into the base of a sterile Petri dish. Prepare two plates per dilution.
- Dispense approximately 15 ml of agar which has cooled to around 44-47°C.
- Mix thoroughly and leave to set.
 - ISO 15214 protocol (MRS pH 5,7):**
Incubate at 30 ±1°C for (72+/-3) hours under aerobic atmosphere. Surface inoculation combined with incubation in an anaerobic or microaerobic atmosphere can be used instead of the pour-plate method.
Note: a double layer of MRS medium can be used (4ml) to enhance growth of *Lactobacillus* and improved reading of colonies
 - NF V04-503 protocol (MRS pH 5,7):**
Incubate at 25 ±1°C for (72+/-3) hours under aerobic atmosphere.
 - ISO 7889 protocol (MRS pH 5,4):**
Incubate at 37 ±1°C for (72+/-3) hours under anaerobic atmosphere.
 - or follow the validated procedure by the laboratory.
- After incubation, count the colonies. Refer to the chosen reference method.

READING AND INTERPRETATION

Colonies of lactic acid bacteria are generally opaque, smooth or sometimes granular, round, with an off-white color.

In some cases and for certain products, it may be necessary to confirm the identification of the colonies by Gram staining or catalase.

QUALITY CONTROL

MRS agar is designed and developed to meet the strictest quality requirements.

The results of the strains tested in the batch by batch quality control are given on the quality control certificate available on request.

LIMITATIONS OF THE METHOD

MRS agar has been evaluated on the main food products and on a large number of bacterial strains. Given the wide variety of food products, manufacturing procedures and microbial flora, it may be necessary to check that the MRS agar is well adapted to the specificity of your products.

When *Lactobacillus* are associated with other interfering flora, it is best to use a more selective medium such as Rogosa agar.

WASTE DISPOSAL

Unused reagents may be considered as non hazardous waste and disposed of accordingly.

Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.








It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and

degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

- de MAN, ROGOSA, SHARPE, - de MAN, ROGOSA and SHARPE (MRS) agar - International Journal of Food Microbiology - 1987, 5 - 227, 232.
- NF ISO 15214 - 1998 – Horizontal method for the enumeration of mesophilic lactic bacteria. Colony-count technique at 30°C.
- ISO 7889. Yogurt (IDF 117) –Enumeration of characteristic microorganisms- Colony-count technique at 37°C.
- NF V 04-503 - Septembre 1988 - Viandes et produits à base de viande - Dénombrement des Bactéries lactiques.

INDEX OF SYMBOLS

| Symbol | Meaning |
|---|------------------------------|
|  | Catalogue number |
|  | Manufacturer |
|  | Temperature limit |
|  | Use by |
|  | Batch code |
|  | Consult Instructions for Use |
|  | Keep away from light |


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