

SUGAR FREE AGAR*For microbiological control only*

Agar for the enumeration of contaminating microorganisms in dairy products

SUMMARY AND EXPLANATION

Sugar free agar is recommended for the detection and enumeration of contaminating microorganisms in butter and other dairy products. Its formulation complies with the one recommended by F.I.L. and I.D.F. (1, 2)

PRINCIPLE

Contaminating microorganisms are those that are not used in fermentation processes during the preparation of dairy products or that do not belong to the specific flora. The enumeration of these microorganisms gives information on the level of contamination of the product tested. To carry out a selective enumeration of these microorganisms, the medium do not contain fermentable carbohydrates, usable by the specific flora as *Lactobacillus*.

CONTENT OF THE KIT

Ready-to-use medium	
REF AEB621836	Pack of 6 bottles of 100 ml
	SFA*

*: Printed on the container

COMPOSITION**Theoretical formula.**

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

Peptone from gelatin.....	7.50 g
Tryptone.....	7.50 g
Sodium chloride.....	5.00 g
Agar.....	14.00 g
Purified water.....	1000 ml

pH : 7.6

REAGENT AND MATERIAL REQUIRED BUT NOT PROVIDED**Reagent:**

- Sterile Ringer (1/4) solution

Material:

- Bacteriology incubator.
- Water baths
- Sterile or aseptic Petri plates

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 reagents if the packaging is damaged.
- Do not use bottles which show signs of contamination.
- Before use, make sure the tamper-proof seal on the bottle screw caps is intact
- The medium must 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 at 2 - 25°C in their box until the expiry date.**

SPECIMENS

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

INSTRUCTIONS FOR USE**Preparation**

1. **Allow the bottles to come to room temperature.**
2. Loosen the cap on the container of agar.
3. Place the bottle of agar in a water bath equipped with a security system set at approximately 50°C. Increase the temperature to 100°C and leave the agar to melt (approximately 20-30 minutes).
4. Screw the cap back on (wear protective gloves to avoid thermal shock) and then mix.
5. 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, then pour in Petri plates.

Inoculation and incubation

1. Place 2.5 g of the product to be tested in 5 ml of sterile solution of Ringer ¼ diluted.
2. Place in a water bath set at 45°C to melt the sample (butter) and homogenise.
3. When the two phases separated, inoculate 0.2 ml of the aqueous phase or of its decimal dilutions, on the medium poured into sterile Petri plates.
4. Incubate at 30°C for 48 hours, then incubate at 20°C for 48 hours.

READING AND INTERPRETATION

Carry out the enumeration taking into account the dilution inoculated.

Do not count pin-point colonies as those are lactic acid bacteria.

QUALITY CONTROL

The Sugar free agar has been designed and developed to meet the strictest quality requirements.

The results obtained using strains tested during controls for bacteriological activity are shown on the quality control certificate for each batch, available from our website (www.biomerieux.com).

LIMITATIONS OF THE METHOD

It is necessary to control in parallel the absence of fermentable carbohydrates in the medium by inoculating it with a fresh culture of *Enterobacter aerogenes*, and then incubate at 37°C for 6 hours. Yeasts that can be part of the specific microflora of some fermented milks, have not to be considered as contaminating microorganisms.

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

1. F.I.L.-I.D.F. 30 : 1964. Numération des organismes de contamination du beurre.
2. F.I.L.-I.D.F. 66 : 1971. Laits fermentés : dénombrement des organismes microbiens de contamination. (Méthode de référence).

INDEX OF SYMBOLS

Symbol	Meaning
	Catalogue number
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use

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