



## Sugar Free Agar

M307

Sugar Free Agar is recommended for the examination of butter in accordance with International Dairy Federation.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	7.500
Pancreatic digest of gelatin	7.500
Sodium chloride	5.000
Agar	14.000
Final pH ( at 25°C)	7.6±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Suspend 34 grams 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. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Butter may be contaminated in the manufacturing process, from contaminated water sources, air or unsanitary equipment. Yeast, moulds and coliforms do not survive pasteurization and are rarely found in butter made under sanitary conditions. Estimates of the number of coliforms, yeasts and moulds or Enterococci in samples of butter taken at various stages of processing are useful in tracing the source of contamination (1). Sugar Free Agar is prepared as recommended by the International Dairy Federation (2) and is based on the formulation developed by Ritter and Eschmann (3) for the detection and enumeration of contaminating bacteria in butter and other processed dairy products.

The medium contains pancreatic digest of gelatin and casein enzymic hydrolysate which provide essential nutrients to the microorganisms. The medium is free of any fermentable carbohydrate and has relatively little nutrient value. Therefore microorganisms that do not form the normal flora of butter and other processed milk products can be grown selectively on this medium. Psychrotrophic and mesophilic gram-negative bacteria also grow well on this medium. Some Lancefield group D streptococci are not able to grow on this medium and hence do not interfere in the total count of the gram-negative bacteria (3). This medium aids differentiating non-lactic acid contaminants from lactic acid bacteria.

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.4% Agar gel

#### Colour and Clarity of prepared medium

Medium amber coloured clear to slightly opalescent gel forms in Petri plates

#### Reaction

Reaction of 3.4% w/v aqueous solution at 25°C. pH : 7.6±0.2

#### pH

7.40-7.80

#### Cultural Response

M307: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
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#### Cultural Response

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<i>Escherichia coli</i> ATCC 25922	50-100	good-luxuriant	$\geq 70\%$
<i>Bacillus cereus</i> ATCC 10876	50-100	good-luxuriant	$\geq 70\%$
<i>Candida albicans</i> ATCC 10231	50-100	good-luxuriant	$\geq 70\%$
<i>Enterococcus faecalis</i> ATCC 29212	50-100	good-luxuriant	$\geq 70\%$
<i>Leuconostoc mesenteroides</i> ATCC 8293	50-100	good-luxuriant	$\geq 50\%$

## Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

## Reference

1. International Dairy Federation, 1964, International Standards FIL-IDF 30
2. Ritter P. and Eschmann K. H., 1966, Alimenta, 5 (2): 433. Thomas S. B., 1969, J. Appl. Bacteriol., 32: 269

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