



## AK Agar No.2 (Sporulating Agar) (Arret and Kirshbaum Medium)

M234

AK Agar No.2 is a culture medium recommended for the production of spores of *Bacillus subtilis* ATCC 6633, which are used for detection of Penicillin and other antibiotic residues in milk and dairy products.

### Composition\*\*

Ingredients	Gms / Litre
Pancreatic digest of gelatin	6.000
Casein enzymic hydrolysate	4.000
Yeast extract	3.000
Beef extract	1.500
Dextrose	1.000
Manganous sulphate	0.300
Agar	15.000
Final pH ( at 25°C)	6.6±0.2

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

### Directions

Suspend 30.8 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 300 ml amounts in Roux or other suitable bottles. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Note : Do not autoclave till the medium has been completely dissolved.

### Principle And Interpretation

Detection of penicillin and other antibiotic residues in milk is of primary importance in the dairy industry. This is done by the Penicillin Milk Test procedure (1). AK Agar formulated by Arret and Kirshbaum is used for the production of spores of *Bacillus subtilis* ATCC 6633, which is used in the Penicillin Milk Test procedure. This medium is highly nutritious due to the presence of yeast extract, beef extract, pancreatic digest of gelatin and casein enzymic hydrolysate in addition to being a source of vitamins and essential amino acids. Dextrose serves as the source of energy as well as the fermentable carbohydrate. Manganous sulphate stimulates sporulation.

A fresh slant culture of *Bacillus subtilis* is washed with sterile physiological saline onto the surface of Roux bottles containing 300 ml sterile medium. The bottles are incubated at 35°C for 5 days and the resulting growth is suspended into 50 ml of sterile physiological saline. The growth is washed by centrifuging the suspension and discarding the supernatant. The sediment obtained is re-suspended in fresh sterile saline and heated at 70°C for 30 minutes to kill vegetative cells and obtain the spore suspension. This spore suspension can be stored for months for use in detection of penicillin/ antibiotic residues in milk and dairy products (2).

### Quality Control

#### Appearance

Cream to yellow homogeneous free flowing powder

#### Gelling

Firm, comparable with 1.5% Agar gel

#### Colour and Clarity of prepared medium

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

#### Reaction

Reaction of 3.08% w/v aqueous solution at 25°C. pH : 6.6±0.2

#### pH

6.40-6.80

#### Cultural Response

Please refer disclaimer Overleaf.

M234: Cultural characteristics observed after an incubation at 35-37°C for 5 days.

Organism	Inoculum (CFU)	Growth	Recovery	Sporulation
<b>Cultural Response</b>				
<i>Bacillus megaterium</i> ATCC 25848	50-100	good-luxuriant	>=70%	positive
<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	>=70%	positive

### 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. Arret and Kirshbaum, 1959, J. Milk and Food Tech., 22:329.
2. Richardson (Ed.), 1995, Standard Methods for the Examination of Dairy Products, 15th Ed., APHA, Washington D.C.

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### Disclaimer :

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