



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

M234

### Intended Use:

Recommended for production of spores of *Bacillus subtilis* ATCC 6633

### Composition\*\*

Ingredients	Gms / Litre
Gelatin peptone	6.000
Tryptone	4.000
Yeast extract	3.000
HM peptone B #	1.500
Dextrose (Glucose)	1.000
Manganous sulphate	0.300
Agar	15.000
Final pH ( at 25°C)	6.6±0.2

# Equivalent to Beef extract

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

### Directions

Suspend 30.8 grams in 1000 ml purified / 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. Cool to 45-50° C.

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, HM peptone B, gelatin peptone and tryptone 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).

### Type of specimen

Pure isolate

### Specimen Collection and Handling

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## Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations :

1.This medium is general purpose medium and may not support the growth of fastidious organisms.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## 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

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

Organism	Inoculum (CFU)	Growth	Recovery	Sporulation
<i>Bacillus megaterium</i> ATCC 25848	50-100	good-luxuriant	≥70%	positive
<i>Bacillus subtilis</i> ATCC 6633 (00003*)	50-100	good-luxuriant	≥70%	positive

Key : \*Corresponding WDCM numbers.

## 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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