# 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 (4).

## 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 |
|---|---|---|---|---|
| *Bacillus megaterium ATCC 25848* | 50-100 | good-luxuriant | >=70% | positive |
| *Bacillus subtilis subsp. spizizenii ATCC 6633* (00003*) | 50-100 | good-luxuriant | >=70% | positive |

Key : *Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

## Reference

1. Arret and Kirshbaum, 1959, J. Milk and Food Tech., 22:329.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

4. Richardson (Ed.), 1995, Standard Methods for the Examination of Dairy Products,15th Ed., APHA, Washington D.C.