

Technical Information

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

Product Code: DM 1234

Application: AK Agar No.2 culture medium is 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

Principle & Interpretation

Detection of penicillin and other antibiotic residues in milk is of utmost importance in the dairy industry. This is done by the Penicillin Milk Test procedure for which AK Agar formulated by Arret and Kirshbaum ⁽¹⁾ is used for the production of spores of *Bacillus subtilis* ATCC 6633, which is subsequently 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 which also serve as a source of vitamins and essential amino acids. Dextrose acts 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 growth is harvested with 50 ml of sterile physiological saline which is separated by centrifuging the suspension. The sediment 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⁾.

Methodology

Suspend 30.8 grams of powder media in 1000 ml distilled water. Shake well and 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 (12 1°C) for 15 minutes. **Note: Do not autoclave till the medium has been completely dissolved.**

Quality Control

Physical 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



Dehydrated Culture Media
Bases / Media Supplements

pH Range:- 6.40-6.80

Cultural Response/Characteristics

DM 1234: Cultural characteristics observed after an incubation at 35°C for 5 days.

Organism	Inoculum (CFU)	Growth	Recovery	Sporuation
<i>Bacillus megaterium</i> ATCC25848	50-100	good-luxuriant	>=70%>	Postive
<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant	>=70%	Postive

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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