

Sporulating Agar (USP Antibiotic Medium 32)

Art. No. 01-069

Also known as

AK 2 Agar; Arret & Kirschbaum Agar, AK Agar; USP Antibiotic Medium 32

Specification

This formulation is according to the Arret and Kirshbaum formulation, adopted by US, FDA for the preparation of spore suspensions for antibiotic assay.

Formula* in g/L

Gelatin peptone.....	6,00
Casein peptone.....	4,00
Yeast extract.....	3,00
Meat extract.....	1,50
Dextrose.....	1,00
Manganous sulfate.....	0,30
Agar.....	15,00
Final pH 6,6 ± 0,2 at 25°C	

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 30,8 g of powder in 1 L of distilled water. Bring it to the boil stirring constantly. Dispense in suitable containers and sterilize in the autoclave at 121°C for 15 minutes.

Description

Sporulation Agar is made according to the original formulation of Arret and Kirshbaum, and later adopted by the FDA for the preparation of *Bacillus subtilis* ATCC 6633 spore suspensions.

To prepare the suspension, suspend growth in 10 mL of 1/4 Ringer's Solution (Art. No. 06-073). With this suspension, inoculate the surface of a Roux flask containing 300 mL of solidified and controlled Sporulation Agar.

The Roux flask must be incubated at 35°C for 5 days. Growth is harvested with 50 mL of sterile Ringer's Solution, aided by a Drigalsky loop or sterile crystal balls (pearls) if necessary. The suspension obtained is carefully centrifuged at 5000 rpm for 15 minutes. Discard the supernatant liquid and suspend the pellet in a fresh volume of Ringer's Solution. Put it in a boiling water bath at 70°C for 30 minutes. This suspension is active for up to 6 months if refrigerated. Depending on the objective of the experiment, it can be standardized by turbidimetry.

References

- ARRET & KIRSCHBAUM (1959) J. Milk Food Tech. 22:329.
- SANCHO, GUINEA, PARES (1980) Microbiología Analítica Básica. Ed. JIM. Barcelona.
- USP 31 - NF 26 (2008) <81> Antibiotic Microbial Assays. US Pharmacopeial Convention Inc. Rockville. MD.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 48 h - 5 days

Inoculum: Inoculate the entire agar surface

Microorganism	Growth	Remarks
<i>Bacillus subtilis</i> ATCC 6633	Good	Spore present
<i>Bacillus cereus</i> var. <i>mycoides</i> ATCC 11778	Good	Spore present
<i>Bacillus cereus</i> ATCC 10876	Good	Spore present
<i>Staphylococcus aureus</i> ATCC 25923	Good	-
<i>Escherichia coli</i> ATCC 8739	Good	-