AK Agar #2 (Sporulating Agar)

Intended Use

AK Agar #2 (Sporulating Agar) is a culture medium for the preparation of spore suspensions for use in procedures for the detection of antibiotic residues in milk and dairy products.

Summary and Explanation

AK Agar #2 was devised by Arret and Kirshbaum for specific use in the production of spores of *Bacillus subtilis* ATCC[™] 6633 for use in the Penicillin Milk Test procedure.¹ This medium was formerly specified in the spore preparation phase of the American Public Health Association disc assay procedure for the detection of sulfa drugs and antibiotics in milk.²

Principles of the Procedure

The peptones and beef extract are sources of nitrogen, sulfur, amino acids and essential trace ingredients. Yeast extract is a rich source of B vitamins. Dextrose is an energy source for bacterial replication. Manganous sulfate plays an important role in the sporulation process.

Formula

BBL[™] AK Agar #2

Approximate Formula* Per Liter

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Pan	creatic Digest of Gelatin	6.0	g
Pan	creatic Digest of Casein	4.0	g
Yea	st Extract		g
Bee	f Extract	1.5	g
De>	trose	1.0	g
Aga	ar	15.0	ğ
Ma	nganous Sulfate	0.3	g
	usted and/or supplemented as required to meet performance criteria.		5

Directions for Preparation from Dehydrated Product

- 1. Suspend 30.8 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- 3. Dispense and autoclave at 121°C for 15 minutes.
- 4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. For preparation of spore suspensions for use in the FDA procedure for the Penicillin Milk Test.¹

Transfer cultures of *Bacillus subtilis* ATCC 6633 monthly to fresh Seed Agar slants. Wash the growth from a fresh slant culture with sterile physiological saline onto the surface of a Roux bottle containing 300 mL of AK Agar #2. Incubate the bottles for 5 days at $35 \pm 2^{\circ}$ C and wash off the resulting growth into 50 mL of sterile physiological saline. Centrifuge the suspension and decant and discard the supernatant fluid. Resuspend the sediment in sterile saline and heat shock the suspension at 70°C for 30 minutes. The resultant spore suspension can be stored for several months. Consult the reference for the test procedure utilizing this *B. subtilis* spore suspension.¹

2. For preparation of spore suspension for use in the APHA procedure for detection of sulfa drugs and antibiotics in milk.² Transfer cells of Bacillus megaterium ATCC 9855 by streaking the entire surface of sterile AK Agar #2 contained in a prescription (180 mL capacity) or Roux bottle. Incubate inoculated bottles at $35 \pm 2^{\circ}$ C for 48 hours. After incubation, wash the spores and vegetative cells from the agar surface with buffered MS (microbiologically suitable) water. Sediment the spores and cells by centrifugation at 5,000 × g for 15 minutes at 3°C. Store the spore suspension in buffered MS water under refrigeration. Consult the reference for the test procedure utilizing this *B. megaterium* spore suspension.²

User Quality Control

Identity Specifications BBL[™] AK Agar #2

DDL AR Agai #2	
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.08% solution, soluble in purified water upon boiling. Solution is light to medium,
	vellow to tan, clear to moderately hazy.
	yenow to tan, clear to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to moderately hazy.
Reaction of 3.08%	. ,
Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response BBL[™] AK Agar #2

Prepare the medium per label directions. Inoculate plates and incubate at $35 \pm 2^{\circ}$ C for 18-24 hours. Reincubate plates at $35 \pm 2^{\circ}$ C and prepare slides after 2 days (and again after 5 days for *B. subtilis* only).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	SPORE PRODUCTION
Bacillus megaterium	9855	10 ² -10 ³	Good	+
Bacillus subtilis	6633	10 ² -10 ³	Good	+

Expected Results

Suspensions containing large numbers of bacterial spores are obtained with the use of AK Agar #2.

References

- 1. Arret and Kirshbaum. 1959. J. Milk Food Technol. 22:329.
- Richardson (ed.). 1985. Standard methods for the examination of dairy products, 15th ed. American Public Health Association. Washington, D.C.

Availability

BBL[™] AK Agar #2 (Sporulating Agar)

Cat. No. 210912 Dehydrated – 500 g

