IFU

Instructions for Use

AK #2 AGAR

Cat. no. U189	AK #2 Agar, 8oz. Glass Bottle, 200ml	12 bottles/box

INTENDED USE

Hardy Diagnostics AK #2 Agar is recommended for use as a culture medium for the preparation of spore suspensions used in the detection of antibiotic residues (such as penicillin and sulfa drugs) in milk and dairy products.

SUMMARY

Arret and Kirshbaum developed AK #2 Agar for use in the production of spores of *Bacillus spizizenii*, ATCC® 6633 for use in the Penicillin Milk Test.(3) The medium is also detailed in the spore preparation phase of the American Public Health Association (APHA) disk assay procedure for the detection of sulfa drugs and antibiotics in milk.(1)

Hardy Diagnostics' AK #2 Agar contains peptones and beef extract for nitrogen, sulfur, amino acids, essential vitamins and minerals. Yeast extract is a source of B vitamins. Dextrose provides the energy source for replication and manganous sulfate is important in promoting sporulation.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Gelatin	6.0gm
Pancreatic Digest of Casein	4.0gm
Yeast Extract	3.0gm
Beef Extract	1.5gm
Dextrose	1.0gm
Manganous Sulfate	0.3gm
Agar	15.0gm

Final pH 6.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

In the preparation of spore suspensions when performing the FDA procedure for the Penicillin Milk Test:[3,4]

1. Transfer a culture of *Bacillus spizizenii*, ATCC® 6633 monthly to a fresh plate or slant of AK #2 Agar and incubate cultures at 35 +/- 2ºC. for 18 hours.

2. Wash the growth with 2 to 5ml of Saline, 0.85% (Cat. no. K59) onto the agar surface and spread the sample across the entire surface of the medium. **Note:** The use of sterile glass beads may facilitate the removal of growth from the agar surface.

3. Incubate the sample for 18-24 hours at 35 +/- 2ºC. and then at room temperature for the remainder of 1 week (6 days).

4. Harvest the spores and cells by washing the growth from the agar surface with 5ml Saline, 0.85% (Cat. no. K59) into a sterile centrifuge tube. Gently swirl the container to loosen the growth, being careful not to break the agar. Sterile glass beads may be used to facilitate the removal of growth from the agar surface.

5. Centrifuge the suspension, and decant and discard the supernatant. Resuspend the sediment in Saline, 0.85% and heat shock the cells at 70ºC. for 30 minutes. The resulting spore suspension can be stored refrigerated for several months. Consult listed reference for testing procedures utilizing the spore suspension.[3]

In the preparation of spore suspension in performing the APHA procedure for detection of sulfa drugs and antibiotics in milk:[1,4]

1. Transfer a culture of *Bacillus megaterium*, ATCC ® 9885 to the surface of AK #2 Agar and streak the inoculum evenly to distribute the cells.

2. Incubate the sample at 35 +/- 2ºC. for 18-24 hours and then at room temperature for the remainder of 1 week (6 days).

3. Harvest the spores and cells by washing the growth from the agar surface with 2 to 5ml of Butterfield's Phosphate Buffer (Cat. no. K109). Gently swirl the container to loosen the growth, being careful not to break the agar. **Note:** The use of sterile glass beads may facilitate the removal of growth from the agar surface.

4. Aseptically transfer the suspension into a sterile centrifuge tube and heat the tube in a boiling waterbath (100ºC.) for 10 minutes.

5. Centrifuge the suspension at 5ºC. for 20 minutes at 20,000 x G and decant the supernatant. Wash the sediment and resuspend it using fresh Butterfield's Phosphate Buffer and centrifuge again under the same parameters. Repeat the wash step two more times.

6. Store the suspension in Butterfield's Phosphate Buffer and refrigerate until use. Consult listed reference for the proper procedure before use.(1)

INTERPRETATION OF RESULTS

Consult listed references for more information.(1,3) It is expected that AK #2 Agar will yield large numbers of bacterial spores.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, saline 0.85% (Cat. no. K59), Butterfield's Phosphate Buffer (Cat. no. K109), incinerators, waterbath, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Bacillus spizizenii ATCC® 6633	A	18-48hr	35°C	Aerobic	Growth

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared*

Microbiological Culture Media for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

AK #2 Agar should appear clear with a slight opalescence, and light to medium amber color.

REFERENCES

1. American Public Health Association. 1993. *Standard Methods for the Examination of Dairy Products*, 16th ed. APHA, Washington, D.C.

2. Anderson, N.L., et al. 2005. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

3. Arret, B. and A. Kirshbaum. 1959. A Rapid Disc Assay Method for Detecting Penicillin in Milk. *J. Milk Food Technol.*; 22:329-331.

4. Dey, B.P., C.A. White, R.H. Reamer and N.H. Thaker. 1998. USDA/FSIS Microbiology Laboratory Guidebook, 3rd ed. www.fsis.usda.gov/science/microbiological_Lab_Guidebook/.

5. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]