BRUCELLA AGAR WITH HEMIN AND VITAMIN K (H & K)

Cat. no. A30	Brucella Agar with H & K,15x100mm Plate, 19ml	10 plates/bag
Cat. no. W23	Brucella Agar with H & K,15x100mm Plate, 26ml	10 plates/bag
Cat. no. H05	Brucella Agar with H & K, 15x150mm Plate, 69ml	10 plates/bag
Cat. no. J74	Brucella Agar with H & K / Anaerobic PEA, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J87	Brucella Agar with H & K / LKV Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Brucella Agar with H & K is recommended for use in the primary isolation and cultivation of anaerobic microorganisms.

SUMMARY

Brucella Agar with H & K is modification of the formulation given by the American Society for Microbiology (ASM). [4] According to Finegold, this media is reported preferable to Heart Infusion Blood Agar Base for the cultivation of anaerobic bacteria. [2] Onderdonk, et al., and Weinstein, et al., both reported the addition of Hemin. [7,10] Summanen, et al., described supplementing the medium with vitamin K. [9] The incorporation of these additives, into Brucella Agar with H & K, enhances the cultivation of some species of anaerobes. [6]

Brucella Agar with H & K is a rich nutrient base that supports the growth of fastidious organisms. The media contains dextrose which provides an energy source; peptones to provide nitrogenous compounds, and yeast extract to supply B vitamins. Growth factors required by some anaerobic bacteria are provided for in the added sheep blood, which also allows the demonstration of hemolytic reactions. Hemin and vitamin K are incorporated into the medium to enhance the growth of gram-positive spore-formers and *Bacteroides* species. [6]

FORMULA

Ingredients per 950ml of deionized water:*

Pancreatic Digest of Casein	10.0gm
Pancreatic Digest of Animal Tissue	10.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm

Dextrose	1.0gm
Sodium Bisulfite	0.1gm
Hemin	10.0mg
Vitamin K	10.0mg
Sheep Blood, Defibrinated	50.0ml
Agar	15.0gm

Final pH 7.0 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating 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 quality control incubation times.

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 *in vitro* diagnostic 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

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

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection. [1-5] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

When possible the clinical specimen should be inoculated directly onto the medium to prevent loss of organism viability. A liquid specimen may be directly applied to the agar surface and streaked with a sterile inoculating loop. Specimens for anaerobic culture should be plated on both non-selective and selective media.

Method of Use: Consult listed references for the correct inoculation procedure. [1-5,9] Incubate plates anaerobically at 35-37ºC. for up to 72 hours. Confirmation of anaerobic organisms should be performed by subculturing to an aerobic Blood Agar plate (Cat. no. A10). Examine anaerobic colonies for hemolytic reaction, colony morphology, gram stain, and further biochemical testing.

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of anaerobic species. [1-5,9]

LIMITATIONS

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

It is suggested that Brucella Agar with H & K plates be reduced, prior to use, for a minimum of 24 hours by placing them in an anaerobe jar at room temperature.

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, slides, anaerobe jars or bags, anaerobic generators and indicators, incinerators, microscopes, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Bacteroides fragilis ATCC ® 25285	A	24-48hr	35°C	Anaerobic	Growth
Bacteroides levii ATCC ® 29147	A	24-48hr	35°C	Anaerobic	Growth
Clostridium perfringens ATCC ® 13124	A	24-48hr	35°C	Anaerobic	Growth; beta-hemolysis
Fusobacterium nucleatum ATCC ® 25586	A	24-48hr	35°C	Anaerobic	Growth
Peptostreptococcus anaerobius ATCC ® 27337	A	24-48hr	35°C	Anaerobic	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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

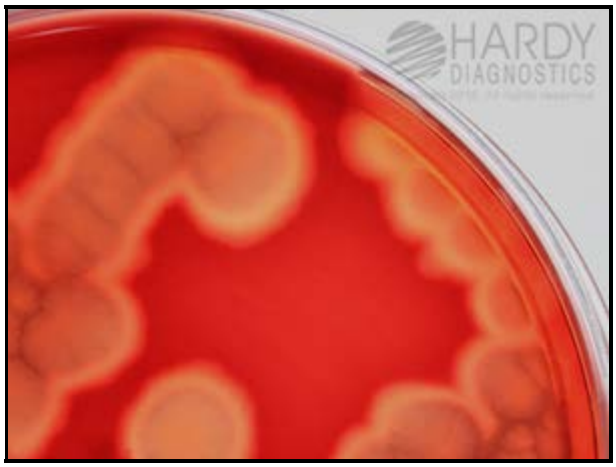
PHYSICAL APPEARANCE

Brucella Agar with H & K should appear opaque, and cherry red in color.

Fusobacterium nucleatum (ATCC ® 25586) colonies growing on Brucella Agar with H and K (Cat. no. A30). Incubated anaerobically for 24 hours at 35ºC.

Bacteroides fragilis (ATCC ® 25285) colonies growing on Brucella Agar with H and K (Cat. no. A30). Incubated anaerobically for 24 hours at 35ºC.

Bacteroides levii (ATCC ® 27337) colonies growing on Brucella Agar with H and K (Cat. no. A30) Incubated anaerobically for 24 hours at 35ºC.



Clostridium perfringens (ATCC ® 13124) colonies growing on Brucella Agar with H and K (Cat. no. A30). Incubated anaerobically for 48 hours at 35ºC.

Peptostreptococcus anaerobius (ATCC ® 27377) colonies growing on Brucella Agar with H and K (Cat. no. A30). Incubated anaerobically for 48 hours at 35ºC.

Uninoculated plate of Brucella Agar with H and K (Cat. no. A30).

REFERENCES

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

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3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology,* J.B. Lippincott Company, Philadelphia, PA.

6. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

7. Onderdonk, A.B., et al. 1974. *Infect. Immun.*; 10:1256.

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

9. Summanen, P., et al. 1993. *Wadsworth Anaerobic Bacteriology Manual*, 5th ed. Star Publishing Company, Belmont, CA.

10. Weinstein, W.M., et al. 1974. *Infect. Immun.*; 10:1250.

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

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