



## Instructions for Use

# WILKINS-CHALGREN MEDIA

Cat. no. <a href="#">G89</a>	Wilkins-Chalgren Agar, 15x100mm Plate, 18ml	10 plates/bag
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## INTENDED USE

Hardy Diagnostics Wilkins-Chalgren Agar is recommended for the cultivation and isolation of anaerobic microorganisms and for the preparation of agar dilution tests. <sup>(3,5,10)</sup>

## SUMMARY

Anaerobic bacteria of clinical significance are known to cause a variety of human infections, including endocarditis, meningitis, wound infections, and bacteremia. Their successful culture is dependent upon strict adherence to their atmospheric requirements, nutritional needs, and appropriate collection and culture constraints. <sup>(2,4,7,8)</sup> Wilkins-Chalgren Agar was established by Wilkins and Chalgren in the mid 1970s as a standard medium for use in determining the minimal inhibitory concentration (MIC) of antibiotics used for anaerobic bacteria by the agar dilution method. The medium was developed to support the growth of most clinically isolated anaerobic microorganisms, without the addition of blood. More recent editions of the CLSI (formerly NCCLS) standard reference method for antimicrobial susceptibility testing of anaerobic bacteria have replaced this medium with the Wadsworth method. <sup>(6)</sup>

Wilkins-Chalgren Agar contains yeast extract to supply essential vitamins, purines, and pyrimidines to enhance the growth of *Prevotella melaninogenica*, and sodium pyruvate to support the growth of *Peptostreptococcus anaerobius* and assacharolytic organisms such as *Veillonella* spp. Arginine is added to improve the growth of *Eubacterium lentum*. Dextrose is added as an energy source. Hemin and vitamin K support the growth of organisms in the *Bacteroides fragilis* group, along with *Prevotella melaninogenica*. Sodium chloride is an isotonic agent and agar is the solidifying agent. <sup>(2,4,7,8)</sup>

## FORMULA

Ingredients per liter of deionized water:\*

Enzymatic Digest of Casein	10.0gm
Enzymatic Digest of Gelatin	10.0gm
Yeast Extract	5.0gm
Sodium Chloride	5.0gm
Dextrose	1.0gm
L-Arginine	1.0gm
Sodium Pyruvate	1.0gm

Hemin	0.005gm
Vitamin K	0.0005gm
Agar	15.0gm

Final pH 7.1 +/- 0.2 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. 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 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](http://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

For a complete discussion on standard methods for testing anaerobic microorganisms, refer to the appropriate procedures as documented in the references. <sup>(3-6,8-10)</sup>

## INTERPRETATION OF RESULTS

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on identification of anaerobes. <sup>(6)</sup>

## LIMITATIONS

*In vitro* susceptibility does not necessarily imply *in vivo* effectiveness.

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, incinerators, 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	
<i>Bacteroides fragilis</i> ATCC® 25285**	A	40-48hr	35°C	Anaerobic	Growth
<i>Bacteroides levii</i> ATCC® 29147	A	40-48hr	35°C	Anaerobic	Growth
<i>Clostridium perfringens</i> ATCC® 13124**	A	40-48hr	35°C	Anaerobic	Growth

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

\*\* Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

## 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

Wilkins-Chalgren Agar should appear clear with a slight opalescence, and light amber in color.

## 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.
2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. Hanson, C.W. and W.J. Martin. 1978. Modified Agar Dilution Method for Rapid Antibiotic Susceptibility Testing of Anaerobic Bacteria. *Antimicro. Agents Chemother.*; 13(3):383-388.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology,

Washington, D.C.

5. Jones, R.N., P.C. Fuchs, C. Thornsberry, and N. Rhodes. 1978. Antimicrobial Susceptibility Tests for Anaerobic Bacteria. Comparison of Wilkins-Chalgren Agar Reference Method and a Microdilution Method, and Determination of Stability of Antimicrobics Frozen in Broth. *Current Microbio.*; Vol. 1:81-83.
6. Jousimies-Somer, H.R., S.P. Citron, D. Baron, E.J. Wexler, and H.M. Finegold. 2002. *Wadsworth-KTL Anaerobic Bacteriology Manual*, 6th ed. Star Publishing Company, New York, N.Y.
7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
8. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
9. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
10. Zabransky, R.J. and K.J. Hauser. 1977. Stability of Antibiotics in Wilkins-Chalgren Anaerobic Susceptibility Testing Medium After Prolonged Storage. *Antimicro. Agents Chemother.*; 12(3):440-441.

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IFU-10847[A]



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