



PRODUCT SPECIFICATION SHEET

Wilkins Chalgren Anaerobic Agar Base (DM292)

Intended Use

Wilkins Chalgren Anaerobic Agar Base (DM292) is recommended for selective isolation, cultivation and susceptibility testing of anaerobes by the agar dilution method.

Product Summary and Explanation

Anaerobic bacteria are widespread in soil, marshes, lake and river sediments, oceans, sewage, food and animals. In humans, anaerobic bacteria normally are prevalent in the oral cavity around the teeth, in the gastrointestinal tract, in the orifices of the genitourinary tract and on the skin. Anaerobic infections in humans and various animals can involve virtually any organ under immunocompromised conditions.⁽¹⁾ Also, anaerobic infections are often associated with tissue necrosis and abscess formation, leading to impaired delivery of antimicrobial agents in blood to the actual site of infection. This explains why anaerobic infections are often aggressively managed with debridement, aspiration and/or surgical removal of infected tissue. Because of the technical and interpretive difficulties associated with anaerobic susceptibility testing, presentation of definitive recommendations is difficult.⁽²⁾ The survival of anaerobic bacteria is dependent on their sensitivity to oxygen, nutritional requirements, appropriate collection, culture medium, and incubation time and temperature.⁽³⁾ Wilkins-Chalgren Agar base was designed by Wilkins and Chalgren⁽⁴⁾ for use in determining the minimal inhibitory concentration (MIC) of antibiotics for anaerobic bacteria by the agar dilution procedure. This medium was recommended in the protocol used in the CLSI Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria.^(5, 6, 7)

More recently, Wilkins-Chalgren Agar has been replaced by Brucella Agar supplemented with laked sheep blood, hemin and vitamin K₃ as the recommended reference medium.⁽⁸⁾ Supplements must be added to these media to support the growth of certain fastidious anaerobes, including *Bacteroides gracilis*, *Bilophila wadsworthia*, *Prevotella* species, *Fusobacterium* species and anaerobic cocci.⁽⁹⁾ The medium can also be supplemented with defibrinated sheep blood, 5% or lysed blood for the growth of fastidious anaerobic bacteria.⁽¹⁰⁾

Principles of the Procedure

Wilkins Chalgren Anaerobic Agar Base contains peptic digest of animal tissues and casein enzymic hydrolysate serve as sources of essential nutrients for microbial metabolism including carbon and nitrogen. Yeast extract provides vitamins and other growth factors like purines and pyrimidines that are essential for the growth of *P.melaninogenica*. Arginine serves as an amino acid source while pyruvate serves as an energy source. Sodium chloride helps to maintain the osmotic balance of the medium. The medium can be made selective for non-sporing anaerobic bacteria and gram-negative anaerobic bacteria by addition of NonSpore Anaerobic Supplement (MS022) and G. N. Spore Anaerobic Supplement (MS017) respectively. Hemin and Menadione (Vitamin K₃) enhances the growth of *Bacteroides* species and *Prevotella melaninogenica*, respectively and many other species of gram-negative anaerobic rods.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Peptic digest of animal tissue	10.00
Yeast extract	5.00
Dextrose	1.00
Sodium chloride	5.00
L-Arginine	1.00





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Sodium pyruvate	1.00
Hemin	0.005
Menadione	0.0005
Agar	10.00
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 43.01 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense and autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix gently and pour into sterile Petri plates.
5. For cultivation of anaerobes, aseptically add the rehydrated contents of 2 vials each of Non-Spore Anaerobic Supplement (MS022) or G. N. Spore Anaerobic Supplement (MS017) as desired to the sterile molten medium before pouring into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Medium amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.3% Solution	pH : 7.1 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.0% Agar gel.

Expected Cultural Response: Cultural characteristics observed with added Non-spore Anaerobic Supplement (MS022) or G.N.Spore Anaerobic Supplement (MS017) under anaerobic condition, after an incubation at 35-37°C of 48 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	<i>Bacteroides fragilis</i> ATCC 25285	50 - 100	good-luxuriant	>=50%
2.	<i>Clostridium perfringens</i> ATCC 12924	50 - 100	good-luxuriant	>=50%
3.	<i>Escherichia coli</i> ATCC 25922	>=10 ³	inhibited	0%
4.	<i>Prevotella melaninogenicus</i> ATCC 15930	50 - 100	good-luxuriant	>=50%

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

For a complete discussion on susceptibility testing of anaerobic bacteria refer to appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for interpretation of results.





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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Wilkins Chalgren Anaerobic Agar Base

Product Code : DM292

Available Pack sizes : 500gm

References

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9. Wexler and Doern. 1995. *In Murray, Baron, Tenover and Tenover (ed.)*. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
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Further Information

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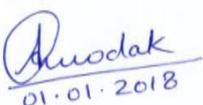
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