



Instructions for Use

anaeroGRO™

Pre-Reduced Anaerobic Culture Media

BACTEROIDES BILE ESCULIN (BBE) AGAR

Cat. no. AG051	Bacteroides Bile Esculin Agar*, Monoplate	1 plate/pouch
Cat. no. AG061	BBE/LKV* Biplate	1 plate/pouch
Cat. no. AG302	DuoPak A, BRU with Hemin and Vitamin K*, Monoplate; BBE/LKV*, Biplate	2 plates/pouch
Cat. no. AG312	DuoPak B, BRU with Hemin and Vitamin K*, Monoplate; BBE/PEA*, Biplate	2 plates/pouch
Cat. no. AG303	MultiPak A, BRU with Hemin and Vitamin K*, Monoplate; PEA*, Monoplate; BBE/LKV*, Biplate	3 plates/pouch

* All AnaeroGRO™ plated media is provided in standard 15x100mm monoplates or biplates. Each plate or set of plates is packaged in an oxygen-free gas flushed foil pouch containing a desiccant and an oxygen scavenger sachet.

INTENDED USE

Hardy Diagnostics AnaeroGRO™ Bacteroides Bile Esculin Agar is an enriched, selective, and differential medium recommended for the isolation and presumptive identification of obligately anaerobic gram-negative bacilli of the *Bacteroides fragilis* group and *Bilophila* spp.

SUMMARY

Livingston et al. developed Bacteroides Bile Esculin Agar to accelerate recognition of the *Bacteroides fragilis* group within 48 hours.^(2,9) The medium was developed by combining the components of 20 percent bile stimulation, esculin hydrolysis, catalase production, and kanamycin inhibition tests. Gentamicin was later substituted for kanamycin because it does not lose potency at incubation temperatures.⁽⁹⁾

BBE Agar is useful for rapid isolation and presumptive identification of the *B. fragilis* group. The medium contains 100ug/ml of gentamicin to inhibit most aerobic organisms and 20% bile to inhibit most other anaerobes. As differential agents, esculin and iron have been incorporated into the agar to aid in detecting the esculin-positive *B. fragilis* group organisms. BBE Agar is also a useful medium for *Bilophila* species, which usually produce black-centered colonies on this medium due to H₂S production. Other organisms that may grow on this medium are occasional strains of *Fusobacterium mortiferum* and *F. varium*, gentamicin-resistant Enterobacteriaceae, enterococci, pseudomonads, staphylococci, and yeast. However, the colony size of the facultative anaerobic organisms is usually less than 1mm in diameter.

AnaeroGRO™ Bacteroides Bile Esculin Agar is packaged in an oxygen-free, reduced state to prevent the formation of toxic oxidized by-products that may damage obligate anaerobes and inhibit the growth of more fastidious species. Culture media that is exposed to environmental oxygen leads to a build-up of reactive oxygen species (ROS) that initiate damaging free radical reactions, which inhibit the growth of anaerobic bacteria. Therefore, ingredients have been added to the AnaeroGRO™ media to neutralize the growth inhibiting effects of peroxide and other reactive oxygen species (ROS) that may develop during the medium's brief exposure to oxygen after it is sterilized and before it is packaged in an oxygen-free environment.

FORMULA

Ingredients per liter of deionized water:*

Tryptic Soy Agar	45.0gm
Oxbile (Oxgall)	20.0gm
Reducing Agents/Peroxide Inhibitors	1.5gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Hemin	12.0mg
Gentamicin	100.0mg

Final pH 7.0 +/- 0.3 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 15-30°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 plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

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.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

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: To ensure optimal recovery of anaerobes, specimens should be protected from air (oxygen) as much as possible during collection, transport, and processing. Consult listed references for instructions concerning collection and transport of anaerobes.^(1,5,6,7,10)

Method of Use: Open the AnaeroGRO™ pouch just prior to use. The agar surface should be dry prior to inoculation. Minimize specimen exposure to ambient oxygen levels in air. Inoculate and streak the medium to obtain isolated colonies. Incubate in an anaerobic atmosphere at 35-37°C. for 18-48 hours. Regardless of atmospheric system used, it is important to confirm anaerobiosis by using an anaerobic indicator, such as resazurin (Cat. no. BR55). Observe for growth and blackening of the medium.

Aerotolerance Testing: Confirmation of obligate anaerobic microorganisms should be performed. A Chocolate Agar plate (Cat. no. E14) incubated in 5-10% CO₂ is required for aerotolerance testing to detect isolates that require CO₂, especially slow-growing, fastidious, facultative or microaerophilic species that do not grow alone on media containing blood (such as *Haemophilus* and *Actinobacillus* spp.). Use of traditional blood agar media alone for CO₂ incubation may yield false-negative results. An additional Blood Agar plate (Cat. no. A10) incubated in air will further detail the atmospheric requirements and hemolytic properties of facultatively anaerobic microorganisms.

INTERPRETATION OF RESULTS

This product is used in conjunction with other biochemical tests to identify cultures of isolated organism. Esculin hydrolysis is indicated by a browning or blackening in the medium surrounding a colony.

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.^(1,3-8,10)

Refer to the *Wadsworth-KTL Anaerobic Bacteriology Manual* or other texts for more information on the identification of anaerobes.⁽⁷⁾

LIMITATIONS

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

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

Some strains of *B. vulgatus*, a member of the *B. fragilis* group, may test esculin-negative.

Esculin can be hydrolyzed by some bile-resistant, non-*B. fragilis* group microorganisms. Some examples of such microorganisms are *Bacteroides splanchnicus*, *Fusobacterium mortiferum*, *Klebsiella pneumoniae*, *Enterococcus* species, and yeasts. In general, members of the *B. fragilis* group are two to three millimeters in size; whereas, the previously mentioned organisms are less than one millimeter in diameter.^(4,8)

Many anaerobes are sensitive to oxygen during the log phase of growth and may be killed by exposure to oxygen before colonies are fully developed. Therefore, it may be necessary to incubate an inoculated culture for 48 hours (three to five days is preferable) before exposing the culture to room air.

Some organisms that should grow on AnaeroGRO™ BBE Agar may be inhibited such as occasional strains of *Fusobacterium mortiferum* and *F. varium*, and gentamicin-resistant strains of Enterobacteriaceae, enterococci, pseudomonads, staphylococci, and yeast. Consequently, it is recommended that a non-selective medium, such as AnaeroGRO™ Brucella Agar with Hemin and Vitamin K (Cat. no. AG301), be inoculated in parallel to ensure growth of all species present.

Failure to cultivate and/or isolate obligate anaerobes may be due to the following:

1. Exposure of specimen to oxygen during transport or processing.
2. Overgrowth of aerobic, facultative organisms, or normal flora. Overgrowth can occur in transport media, Thioglycollate broth, or on non-selective plated media. This can be controlled by avoiding normal flora during specimen collection and by utilizing selective plated media.
3. Leaks in the anaerobic incubation system; e.g. faulty O-rings or vents.
4. Failure to use an anaerobic indicator (such as resazurin) to monitor for complete anaerobiosis.
5. Anaerobic gas mixture contains toxic gas, oxygen; or does not include CO₂, which is necessary for some anaerobes.
6. Failure to use a non-selective medium as part of the primary specimen set-up, since some fastidious anaerobes are inhibited by selective media.
7. Failure to incubate cultures for extended periods of time. Some fastidious anaerobes are slow growers (such as *Porphyromonas* and *Actinomyces* spp.), especially if present in small numbers, and may require 5 to 7 days of incubation in order to be visible on plated media.
8. Exposure of developing colonies on plated media to air, especially when opening jars to check for growth. Anaerobes are most sensitive to oxygen during the log phase of growth. Do not open jars or pouches at less than 48 hours of incubation (except when incubating BBE or EYA plates, since organisms that are selected for on these plates grow rapidly). Some fastidious anaerobes may lose viability with only 15 minutes of exposure to oxygen. Plates incubated in anaerobic chambers or unopened pouches can be inspected at 24 hours, since the plates are not exposed to oxygen during inspection.

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,

incubators incinerators, anaerobic culture materials, such as gas generators (Cat. no. AN25US), chambers, transports (Cat. no. S120D), jars (Cat. no. 16000), and oxygen indicators (Cat. no. BR55), 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	18-24hr	35°C	Anaerobic	Growth; blackening of the media
<i>Proteus mirabilis</i> ATCC® 12453**	B	18-24hr	35°C	Aerobic	Inhibited

* 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

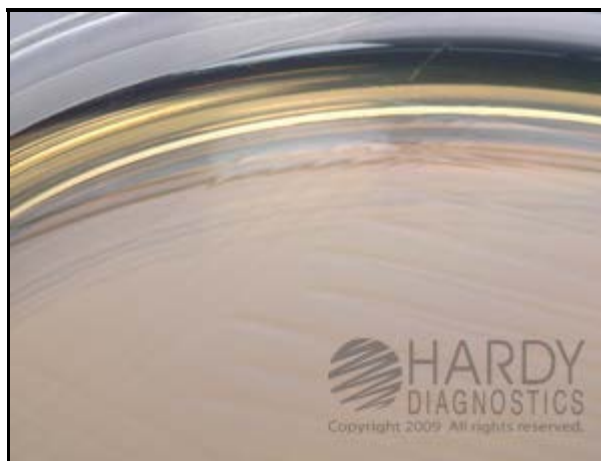
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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

AnaeroGRO™ Bacteroides Bile Esculin (BBE) Agar should appear opalescent, and medium amber with blue tinge in color.



Bacteroides fragilis (ATCC® 25285) colonies growing on AnaeroGRO™ Bacteroides Bile Esculin Agar (Cat.



Proteus mirabilis (ATCC® 12453) growth inhibited on AnaeroGRO™ Bacteroides Bile Esculin Agar (Cat.

no. AG051). Incubated anaerobically for 24 hours at 35°C.

no. AG051). Incubated aerobically for 24 hours at 35°C.

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