Product Insert

BACTEROIDES BILE ESCULIN AGAR (BBE)

Products

AS-114	Bacteroides Bile Esculin Agar (BBE)	1 plate / pkg
AS-144	Bacteroides Bile Esculin Agar (BBE)	4 plates / pkg

The following products contain BBE as one of multiple components

AS-212	BBE/LKV Biplate	1 plate / pkg
AS-242	BBE/LKV Biplate	4 plates / pkg
AS-302	BRU Mono / BBE-LKV Biplate	1 plate each / pkg
AS-322	BRU Mono / BBE-PEA Biplate	1 plate each / pkg
AS-323	BRU Mono / PEA Mono / BBE-LKV Biplate	1 plate each / pkg
AS-444	BRU Mono / PEA Mono / LKV Mono / BBE Mono	1 plate each / pkg

Intended Use

Bacteroides Bile Esculin (BBE) agar is an enriched, selective, and differential medium used for the isolation and presumptive identification of obligately anaerobic gram-negative bacilli of the *Bacteroides fragilis* group and *Bilophila wadsworthia* (4).

Summary

BBE agar contains casein, soy peptones, hemin, and vitamin K_1 as the nutritive base of the media. Selective agents include gentamicin, which inhibits facultative anaerobes, and bile, which inhibits most gram-positive bacteria and anaerobic organisms other than the *B. fragilis* group. The addition of esculin and ferric ammonium citrate to the media permits the recognition of esculin hydrolysis by the organisms. The result is a brown to black coloration around the esculin-positive colonies. *Bilophila* species usually produce black-centered colonies on BBE due to hydrogen sulfide production. Hemin is added as a growth factor and allows testing of catalase production. This media is prepared, stored, and dispensed under oxygen-free conditions to prevent the formation of oxidized products prior to use.

Formulation*

Pancreatic Digest of Casein	15.00	g
Select Soytone	1.00	g
Yeast Extract	5.00	g
Sodium Chloride	5.00	g
Agar	15.00	g
Oxgall	20.00	g
Hemin (0.1% solution)	10.00	mL
Esculin	1.00	g
Ferric Ammonium Citrate	0.50	g
Gentamicin (4.0% solution)	2.50	mL
DI Water	1.00	L

Final pH: 7.1 ± 0.4 at 25° C
Final weight: 16.0 g ± 1.6 g for Mono plates
Final weight: 8.0 g ± 0.8 g for Bi-plates

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

Precautions

For *IN VITRO DIAGNOSTIC USE* only. Utilize approved biohazard precautions and aseptic technique when using this product. This product is for use only by properly-trained and qualified personnel. Sterilize all biohazard waste prior to disposal.

Storage and Shelf Life

Storage: Upon receipt, store at room temperature in original package until used. Avoid overheating or freezing. Do not use media if there are signs of deterioration (shrinking, cracking, or discoloration due to oxidation of media) or contamination. The expiration date applies to the product in its original packaging and stored as directed. Do not use product past the expiration date shown on the label.

Shelf Life: 90 days from date of manufacture.

Procedure

Specimen Collection: Protect specimens for anaerobic culture from oxygen during collection, transportation, and processing. Consult appropriate references for detailed instructions concerning collection and transportation of anaerobes.

Methods for Use: BBE agar should be inoculated directly with a clinical specimen or from a broth that has been inoculated from a clinical specimen. Streak plates with inoculum to obtain isolated colonies and immediately place in an anaerobic atmosphere, incubating at 35-37°C for 18-48 hours. Extended periods of incubation may be required to recover some anaerobes. Extended incubation time may also result in loss of selectivity of the medium which can result in the overgrowth of organisms that should be inhibited. Detailed instructions for processing anaerobic cultures can be found in the listed references.

Materials Required, But Not Provided

Standard microbiological supplies and equipment such as loops, saline blanks, slides, staining supplies, microscope, incinerator / autoclave, incubators, anaerobic chamber / anaerobic jars, disinfectant, other culture media, and serological / biochemical reagents.

Interpretation of Results

If used properly, BBE agar supports good growth of the *Bacteroides fragilis* group (*B. fragilis, B. distasonis, B. thetaiotaomicron, B. ovatus,* and *B. vulgatus*) and inhibits growth of most facultative anaerobes and most other non-*Bacteroides* obligately anaerobic bacteria. Members of the *B. fragilis* group should grow as brown to black colonies surrounded by a brown to black zone in the media, except for *B. ovatus* and *B. vulgatus,* which produce catalase.

Limitations

BBE agar will not provide complete information for identification of bacterial isolates. Additional test procedures and media are required for complete identification. Some organisms that would normally grow on BBE medium may be inhibited. It is recommended that a non-selective medium, such as Brucella Blood Agar (BRU, catalog #: AS-111) also be inoculated from the same clinical specimen to assure recovery of all species present. Some strains of facultative organisms (which should be inhibited) may grow on BBE. A test for aerotolerance should be performed to confirm that each colony type is an obligate anaerobe. Consult reference materials for additional information.

Quality Control

The following organisms are routinely used for quality control performance testing at Anaerobe Systems.

Organism Tested	ATCC #	Results	Time	Special Reaction
Bacteroides fragilis	25285	Growth	24 hrs	Esculin hydrolysis
Prevotella melaninogenica	25845	No Growth		
Fusobacterium necrophorum	25286	No Growth		
Fusobacterium nucleatum	25586	No Growth		
Clostridium perfringens	13124	No Growth		
Peptostreptococcus anaerobius	27337	No Growth		
Staphylococcus aureus or Enterococcus faecalis	25923 29212	Inhibited to No Growth		
Escherichia coli	25922	Inhibited to No Growth		
Proteus mirabilis	12453	Inhibited to No Growth		
Propionibacterium acnes or Clostridium difficile	6919 9689	No Growth		

User Quality Control: The final determination to the extent and quantity of user laboratory quality control must be determined by the end user.

If sterility testing is to be performed on this product, a representative sample of the lot(s) should be incubated anaerobically and aerobically for 48 – 96 hours.

If the nutritive/inhibitory capacity of this media is to be tested for performance, it is recommended that the following ATCC organisms be evaluated for growth/inhibition.

Organism	ATCC#	Expected Growth	Special Reactions
B. fragilis	25285	24 hrs	Esculin hydrolysis
P. melaninogenica	25845	Inhibited	
F. necrophorum	25286	Inhibited	
C. perfringens	13124	Inhibited	
E. coli	25922	Inhibited	

Physical Appearance: BBE should appear translucent yellow/green in color.

References

1. Dowell, V. R., Jr., G. L. Lombard, F. S. Thompson and A. Y. Armfield. 1977. *Media for the Isolation, Characterization and Identification of Obligately Anaerobic Bacteria.* USDHHS, CDC. Atlanta, GA 30333.

2. Engelkirk, P. G., Duben-Engelkirk, J. and Dowell, V. R. 1992. *Principles and Practices of Clinical Anaerobic Bacteriology*. Star Publishing Co., Belmont, CA 94002.

3. Holdeman, L. V., F. P. Cato and W. E. C. Moore. 1987. *Anaerobe Laboratory Manual*. Virginia Polytechnic Institute and State University. Blacksburg, VA 24061

4. Jousimeis-Somer, H. R., Summanen, P., Citron, D. M., Baron, E. J., Wexler, H. M. and S. M. Finegold. 2002. *Wadsworth – KYL Anaerobic Bacteriology Manual*. Star Publishing Co., Belmont, CA 94002.

5. CLSI. *Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard- Third Edition.* (2004). CLSI document M22-A3. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

Revision Date: 10/16/17