BACTERIODES BILE ESCULIN (BBE) AGAR

- For in vitro use only - Catalogue No. PB10

Our Bacteroides Bile Esculin (BBE) Agar is a primary plating medium used for the selection and presumptive identification of members of the *Bacteroides fragilis* group.

BBE Agar was developed by Livingston, et al, in 1978 to accelerate presumptive identification of the *B. fragilis* group. BBE Agar contains the peptones, pancreatic digest of casein and papaic digest of soybean meal, which provide the organism with nitrogen, amino acids, and other elements important for growth. Hemin is a growth factor that allows for the testing of catalase production. Resistance to bile, and esculin hydrolysis are the characteristics that characterize the *B. fragilis* group. The presence of bile stimulates growth of the *B. fragilis* group while inhibiting most other anaerobic organisms. Esculin is present in the medium to detect the bacterial enzyme, esculinase, which hydrolyzes esculin to release glucose and esculetin as end products; visual detection is possible since esculetin reacts with ferric ammonium citrate in the medium to form a brown-black phenolic iron complex. Selectivity of the medium is increased by the addition of the broad-spectrum antibiotic gentamicin.

Formula per Litre of Medium

Pancreatic Digest of Casein	15.0 g
Papaic Digest of Soybean Meal	5.0 g
Sodium Chloride	5.0 g
Bile	20.0 g
Esculin	1.0 g
Ferric Ammonium Citrate	0.5 g
Hemin	12.5 mg
Agar	15.0 g
Gentamicin	0.1 g

pH 7.0 ± 0.2

Recommended Procedure

1. Allow medium to adjust to room temperature prior to inoculation.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate plates in an inverted position, anaerobically at 35°C.
4. Examine plates after 24 and 48 hours.

Interpretation of Results

A positive presumptive result for the *B. fragilis* group is growth on BBE Agar accompanied by esculin hydrolysis, which is observed as a darkening of the medium. The *B. fragilis* group (99%) show two distinct morphologies on BBE Agar:

1. Circular, raised, entire edge, dull, opaque, charcoal gray colonies surrounded by a dark zone with precipitated bile
2. Circular, raised, entire edge, glistening, butyrous, semi-transparent, light to dark gray colonies surrounded by a gray zone with no precipitate.

A negative result would be no growth on BBE Agar or growth with no darkening of the medium.

If desired, catalase production may also be determined by the addition of 3% hydrogen peroxide to the BBE culture plate and observing for the evolution of gas. The observance of bubbles is a positive test for catalase production, while no evolution of bubbles is a negative test BBE culture plates must be exposed to the atmosphere for 30 minutes prior to the addition of hydrogen peroxide.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Occasionally B. fragilis strains fail to grow because of the concentration of bile in the medium*

- *Some Enterococcus, Klebsiella, Fusobacterium strains may grow on BBE Agar, but are easily distinguished from members of the B. fragilis group due to their smaller size and different colonial morphology*

- *B. vulgatus, a member of the B. fragilis group, gives atypical results since it does not usually hydrolyze esculin*

- *Some non-fragilis Bacteroides spp. such as B. splanchnicus and B. eggerthii are bile resistant, hydrolyze esculin, and grow on BBE Agar, but are rarely isolated*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Bacteroides fragilis ATCC 25285	Growth with blackening of medium
Clostridium perfringens ATCC 13124	Inhibition

Storage and Shelf Life

Our Bacteroides Bile Esculin (BBE) Agar should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 10 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
PB10	BBE Agar [Standard 15x100-mm plate]	10/pkg
PB10B	BBE / BBE Biplate [Biplate 15x100-mm plate]	10/pkg

References

1. Livingston SJ, Kominos SD, Yee RB. New medium for selection and presumptive identification of the *Bacteroides fragilis* group. J Clin Micro 1979; 9(2):448-53.
2. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
3. Balows A, Hausler WJ, Herrman KL et al. Manual of clinical microbiology. 5th ed. Washington: ASM, 1991.
4. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.
5. MacFaddin, JF. Biochemical tests for the identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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