BRUCELLA ANAEROBIC AGAR

- For in vitro use only - Catalogue No. PB85

Our Brucella Anaerobic Agar is a general purpose plating medium used for the isolation and cultivation of anaerobic bacteria.

Brucella Anaerobic Agar is an enriched version of Brucella agar. Two main modifications proved beneficial for the isolation of anaerobes: the addition of hemin was first described by Onderdonk and Weinstein et al; the addition of Vitamin K_1 was subsequently reported by Sutter, Citron, and Finegold. Both modifications resulted in increased growth of some anaerobic bacteria.

The nutritional requirements needed to support growth are provided by the various peptones and extracts contained in the medium. The medium is also supplemented with defibrinated sheep blood to allow for visualization of hemolytic reactions. Brucella Anaerobic Agar is an excellent non-selective medium and can be used as a primary plating medium for a variety of clinical samples. It is important to remember that anaerobic culture media permit facultatively anaerobic bacteria to grow as well. Therefore an aerotolerance test should be performed on all isolates before being designated as anaerobes.

Formula per Litre of Medium

Pancreatic Digest of Casein	10.0 g
Peptic Digest of Animal Tissue	10.0 g
Yeast Extract	2.0 g
Dextrose	1.0 g
Sodium Chloride	5.0 g
Sodium Bisulfite	0.1 g
Agar	15.0 g
Hemin	5.0 mg
Vitamin K_1	10.0 mg
Defibrinated Sheep Blood	50.0 mL

pH 7.0 ± 0.2

Recommended Procedure

1. Prior to inoculation, reduce the medium by placing them overnight under anaerobic conditions at room temperature.
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 48 hours. Further incubation may be required to recover some anaerobes. Primary plates should be incubated 5 to 7 days and examined at regular intervals.

Interpretation of Results

If Brucella Anaerobic Agar is used as a primary plating medium, examine the medium using a stereoscopic microscope or hand lens. Record a detailed description of each colony type including the colonial morphology, and characteristics such as pitting, swarming, hemolysis, and pigment production. These characteristics may help later in identifying the isolate. The primary plate should be kept for 5 to 7 days and an aerotolerance test should be performed on all new isolates.

Colonies of interest should be Gram-stained and sub-cultured onto a non-selective medium so that further tests can be performed from pure culture.

- *Some slow-growing anaerobic bacteria may require up to 7 days of incubation to produce noticeable colonies*

- *During examination, minimize the exposure of culture plates to oxygen as even a 10-min exposure may kill some oxygen-sensitive anaerobes*

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. Plates are incubated anaerobically for 48 hours.

Organism	Expected Result
Clostridium perfringens ATCC 13124	Growth
Bacteroides fragilis ATCC 25285	Growth
Fusobacterium nucleatum ATCC 25586	Growth
Peptostreptococcus anaerobius ATCC 27337	Growth

Storage and Shelf Life

Our Brucella Anaerobic 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 8 weeks from the date of manufacture.

References

1. Weinstein WM, Onderdonk AB, Bartlett JB, Gorbach SL. Experimental intra-abdominal abscesses in rats. I. Development of an animal model. Infect Immun 1974; 10:1250.
2. Onderdonk AB, Weinstein WM, Sullivan NM, Bartlett JG. Experimental intra-abdominal abscesses in rats. II. Quantitative bacteriology in infected animals. Infect Immun 1974; 10:1256
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook. Washington, DC: ASM, 1992.
5. NCCLS. Quality assurance for commercially prepared microbiological culture media. 2nd ed. NCCLS document M22-A2. Wayne, PA: NCCLS, 1996.
6. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.

Original: January 2003
Revised / Reviewed: October 2014