ANAEROBIC LAKED BLOOD AGAR

- For in vitro use only - Catalogue No. PA55

Our Anaerobic Laked Blood Agar is an enriched, general purpose plating medium used for the isolation and cultivation of anaerobic bacteria.

Anaerobic Laked Blood Agar is an enriched version of Brucella Agar. Two modifications were made to Brucella Agar that proved beneficial for the enhanced growth of various *Bacteroides* species: Onderdonk and Weinstein et al. first described the addition of hemin; and the addition of Vitamin K_1 was subsequently reported by Sutter, Citron, and Finegold.

The nutritional requirements needed to support growth are provided by the various peptones and extracts contained in the medium. Anaerobic Laked Blood Agar is also supplemented with laked sheep blood that has shown to enhance pigment production by some pigmented anaerobic bacteria. After incubation, the plates should be examined under long-wave (366-nm) ultraviolet light for the presence of brick-red fluorescence, which is characteristic of the genus *Prevotella* and *Porphyromonas*. This medium is a suitable primary plating medium, but ideally it should be used as a secondary medium for the rapid demonstration of pigment production by *Prevotella* and *Porphyromonas*.

It should be noted that two similar anaerobic formulations are also available:

1. **PB85** Brucella Anaerobic Agar contains 5% defibrinated sheep blood instead of laked blood and therefore allows for the interpretation of hemolytic reactions by anaerobes.

2. **PK15** Kan/Van Anaerobic Agar (KVLB) is the same formulation with antibiotics added. Kanamycin and vancomycin allows for the selective isolation of *Bacteroides* and *Prevotella* spp.

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
Laked Sheep Blood	50.0 mL

pH 7.0 ± 0.2

Recommended Procedure

1. Prior to inoculation, the medium should be reduced immediately by placing them overnight under anaerobic conditions.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies. If the medium is used as a secondary medium, pick pigmented colonies from an appropriate primary plating medium and streak them onto Anaerobic Laked Blood Agar.
3. Incubate plates in an inverted position, anaerobically at 35°C.
4. Examine plates after 48 hours, and under long-wave (366-nm) UV for the presence of fluorescent colonies.
5. After inspecting plates re-incubate plates for 4 days or more to detect slow growers, new morphotypes, or late pigmenters.

Interpretation of Results

After the incubation period, colonies of interest can be tested further. The colonial morphology of the different colony types present should be noted along with any pitting or pigment production observed. As indicated, plates should be examined under UV to determine the presence florescence. Coccobacilliary organisms that fluoresce brick-red or produce black colonies are in the pigmented *Prevotella-Porphyromonas* spp. group.

Additional tests such as gram stain, catalase, spot indole, and special-potency antibiotic disk susceptibility test should also be performed to presumptively identify the anaerobic organisms present.

- *Some slow-growing anaerobic bacteria may require up to 7 days of incubation to produce noticeable colonies*
- *Some Veillonella species also fluoresce red under ultraviolet light*
- *Different species of pigmented Prevotella and Porphyromonas vary in the degree and rapidity of pigment production. The identity of strains not showing pigmentation after the incubation period must be established by other biochemical tests*
- *Brick-red fluorescence is the only reliable color for presumptive identification of pigmented species of Porphyromonas and Prevotella*

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
Prevotella melaninogenica ATCC 25845	Growth Red Fluorescence
Bacteroides fragilis ATCC 25285	Growth No Fluorescence

Storage and Shelf Life

Our Anaerobic Laked Blood 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. Shah HN, Bonnett R, Mateen B, Williams RA. The porphyrin pigmentation of *Bacteroides melaninogenicus*. Biochem J 1979; 180:45-50.
4. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
5. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.

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