Designation: NCIB 8395 [Delft 74]
Deposited Name: Clostridium nigrificans Werkman and Weaver

### Medium
ATCC® Medium 1249: Modified Baar’s medium for sulfate reducers
ATCC® Medium 260: Trypticase soy agar/broth with defibrinated sheep blood

### Growth Conditions
Temperature: 45°C to 55°C
Atmosphere: Anaerobic

### Propagation Procedure
1. Open vial according to enclosed instructions or visit www.atcc.org for instructions.
2. Under anaerobic conditions aseptically rehydrate the entire pellet with approximately 0.5 mL of #1249 broth. Aseptically transfer the entire contents to a 5-6 mL tube of #1249 broth. Additional test tubes can be inoculated by transferring 0.5 mL of the primary broth tube to these secondary broth tubes. Best practice dictates the use of pre-reduced media.
3. Use several drops of the primary broth tube to inoculate a #260 plate and/or #260 agar slant.
4. Incubate in an anaerobic atmosphere at 45 to 55°C for 48 hours. Incubate one agar plate aerobically at 37°C to check for contamination.

**ANAEROBIC CONDITIONS:**
Anaerobic conditions for transfer may be obtained by the use of an anaerobic gas chamber or placement of test tubes under a gassing cannula system connected to anaerobic gas.

Anaerobic conditions for incubation may be obtained by any of the following:
- Loose screw caps on test tubes in an anaerobic chamber
- Loose screw caps on test tubes in an activated anaerobic gas pack jar
- Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained

### Notes
Always use freshly prepared anaerobic media.
Either 100% N₂ or 80% N₂-10% CO₂-10% H₂ can be used as the anaerobic gas for culturing this organism.

Additional information on this culture is available on the ATCC® web site at www.atcc.org.

### References
References and other information relating to this product are available online at www.atcc.org.

### Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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