Designation: MLS10  
Deposited Name: Bacillus selenitireducens Switzer Blum et al.

Medium  
ATCC Medium 2100: Bacillus haloalkaliphile medium

Growth Conditions  
Temperature: 26.0°C  
Atmosphere: Anaerobic

Propagation Procedure  
1. Sterilize the top of the Balch tube by spraying it with 70% ethanol and then flaming the top.  
2. If needed exchange the gas in the test tube for 100% N₂.  
3. If the medium is pink (see discussion about resazurin) add 2.0 ml of reducing agent (3% cysteine, stock solution) per 100 ml of medium. Let the medium sit at room temperature for 10 to 20 minutes, until the resazurin becomes colorless, before inoculating.  
4. Open the freeze-dried vial according to the enclosed instructions. Take a gassed 1.0 ml syringe tipped with 22 gauge needle and withdraw 0.5 ml of medium from the Balch tube and rehydrate the freeze dried pellet. Immediately place the re-hydrated vial under a stream of sterile gas, 80% H₂-20% CO₂ to maintain anaerobicity.  
5. Using the same syringe, withdraw the cell suspension from the vial and transfer it to the Balch tube. Plate 0.1 ml of the inoculated culture onto a non-selective medium and incubate aerobically at 26°C.  
6. Growth should be detected in the broth within 48 to 96 hours. No growth should be detected on the aerobic plate or broth.

ANAEROBIC CONDITIONS:  
A. Balch tubes (available from Bellco Glass, Vineland, NJ; are specially designed for anaerobic work and use an aluminum crimp cap to hold a rubber stopper in place. Needles can easily be inserted through the stopper, and the tubes can be pressurized to 2 atm. Alternatively, serum vials may be used, or screw cap tubes with butyl rubber stoppers, in the latter case the stopper may be removed and the tube placed under a cannula system that dispenses sterile, oxygen free gas for addition of reducing agents or inoculation.  
B. Resazurin is a commonly used redox indicator that is pink when the redox potential is above +50 mv, and colorless when the redox potential is below -110 mv, i.e. highly reducing. Most strict anaerobes require this low redox potential for optimum growth.  
C. To obtain a fully reduced medium, it is necessary that the medium be anoxic and that a reducing agent be added. Common reducing agents are sodium sulfide, cysteine, dithiothreitol, and titanium citrate.  
D. Syringes can be made anaerobic by one of two methods.  
   1. Displace the dead space in the syringe with a sterile  
   2. Displace the dead space in the syringe with a

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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