Designation: COOI3B [DSM 13966]
Deposited Name: *Bacillus subterraneus* Kanso et al.

**Medium**

ATCC® Medium 2252: Bacillus subterraneus medium (MR medium)

**Growth Conditions**

Temperature: 40.0°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. Exchange the gas in the test tube for 80% N₂, 20% CO₂.
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. Using a gassed 1.0 ml syringe tipped with 22 gauge needle, 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, anaerobic gas to maintain anaerobicity.
5. Using the same syringe, withdraw the 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 30°C.
6. Growth should be detected in the broth within 2 to 3 days. 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 reducing agent.

**Notes**

Under microscopic inspection at 1000X cells are mostly straight rods but some are curved. Cells are found singly, in pairs and in chains. On #2252 agar, colonies are irregular, dull, flat, erose, and size variation. This culture will also grow on nonselective media aerobically.

**References**

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

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