ATCC Medium: 2601 Methanocalculus chunghsingensis Medium

Solution A: (100X solution; use 10 ml of solution A per liter of medium)
- 100.0 g Ammonium chloride
- 100.0 g Magnesium chloride hexahydrate
- 40.00 g Calcium chloride dihydrate

Dissolve in deionized water, adjust pH to 4.0 with HCl and bring up to one liter.

Solution B: (500X solution; use 2 ml of solution B per liter of medium)
- 200.0 g Potassium dibasic phosphate trihydrate

Dissolve in deionized water then bring the volume up to one liter.

Solution C: (500X; use 2 ml of solution C per liter of medium)
- 0.5 g Resazurin

Dissolve in deionized water, then bring the volume up to one liter.

Trace Mineral Supplement, Catalog No. MD-TMS: (Available from ATCC as a sterile ready-to-use liquid.)
- 10.0 ml per liter of medium

To prepare the medium dissolve 8.4 gm sodium bicarbonate in 976.0 ml of deionized water (100 mM Na_2HCO_3). The solution is bubbled with an oxygen-free gas mixture of N_2-CO_2 (70:30). While gassing, the stock solutions A and B are added. The high initial pH of this solution may cause the minerals to precipitate. As the pH decreases as a result of equilibration of the CO_2:bicarbonate buffering system the minerals will go back into solution; at the same time O_2 is driven out of the medium. Dry ingredients are also added and dissolved in the order listed.

- 2.0 g Yeast extract
- 2.0 g Trypticase peptones

The medium is allowed to gas until it has become clear, usually 30 to 60 minutes depending on the gas flow rate. The pH of the cleared medium should be between pH 7 and pH 7.3. At this point the solution C and trace minerals supplement can be added to the medium.

Prepare separate solutions for Biologist to add at time of Inoculation

0.5 gm KCl in 10 ml distilled water, filter sterilize
2 M formate
1 M acetate
Co-enzyme M 5 g in 100 ml distilled water, filter sterilize
All solutions should be stored in balsh tubes under anaerobic conditions.
Gas mix 80/20

The medium is then ready to be transferred to culture tubes. For this purpose, a gas-cannula system with at least three separate gassing ports should be used. A rack of Balch tubes should be made ready. The flask of medium is continuously gassed with one gas cannula; the other two cannula are placed, one each, in two sequential Balch tubes. A 10 ml pipette is used to pipette 10 ml of medium from the flask to the 1st Balch tube. The cannula is then removed from this tube and at the same time a butyl rubber stopper is pushed into the top; it is important to do this all in one motion to minimize contamination of the headspace of the tube with air. This cannula is then moved to the 3rd tube in sequence and the 2nd tube is filled with media and capped, and so on. After the all tubes are filled, capped, and crimped, the medium can be autoclaved. Following autoclaving a precipitate may form; with periodic mixing this should go back into solution within 48 hours.

Note: In our experience, the resazurin is often colorless after autoclaving MS medium, probably because the yeast extract provides enough reducing power to cause complete reduction of the dye. We still recommend adding a reducing agent to the medium.

Reducing agents:

We suggest adding the reducing agent to the medium at least one hour before the medium is to be inoculated.

Co-enzyme M (mercaptoethanesulfonic acid) (100 X solution): *Dissolve 5.0 g in 100 ml of deionized water. Distribute into screw cap test tubes, 5–6 ml per tube and seal with rubber stoppers under N_2 gas. Autoclave to sterilize. Excess tubes can be stored at room temperature for up to 2 months. Co-enzyme M is a compound produced by many methanogens. Some methanogens are sensitive to stronger reducing agents such as sodium sulfide. Co-enzyme M is the standard reducing agent we use when working with methanogens.*

Sodium sulfide (100 X solution): *Dissolve 1.5 g in 100 ml of distilled water. Distribute into screw cap test tubes, 5–6 ml per tube, and seal with Hungate stoppers. Autoclave to sterilize. Excess tubes can be stored frozen for up to 6 months. Once thawed a tube of sodium sulfide should not be used for more than a week. CAUTON: if sodium sulfide comes into contact with a strong acid, hydrogen sulfide (H_2S), a very toxic gas is liberated immediately.*

Cysteine (100X solution): *Dissolve 3.0 g in 100 ml of distilled water. Distribute into screw cap test tubes, 5–6 ml per tube, and seal with Hungate stoppers. Autoclave to sterilize. Excess tubes can be stored frozen for up to 6 months. Once thawed, a tube of cysteine should not be used for more than a week.*