

ATCC Medium: 2467 MS-OCM Base Medium

Sodium bicarbonate.....	8.4 g
DI Water.....	976.0 ml

Dissolve sodium bicarbonate in deionized water (100 mM Na₂HCO₃). Bubble solution with an oxygen-free gas mixture of N₂-CO₂ (70:30). While gassing, add 10 ml of Solutions A (see below) and 2 ml of Solution B (see below). The high initial pH of this solution may cause the minerals to precipitate. As the pH decreases as a result of equilibration of the CO₂: bicarbonate buffering system, the minerals will go back into solution; at the same time O₂ is driven out of the medium. Next, add and dissolve dry ingredients in the order listed:

Yeast extract.....	2.0 g
Trypticase peptones.....	2.0 g

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 clear medium should be between pH 7 and pH 7.3. At this point add 2 ml of Solution C (see below) and 10 ml of Trace Minerals Supplement (available from ATCC, cat. MD-TMS) to the medium.

The medium is then ready to be transferred to culture tubes. Dispense with continual gassing. After the all tubes are filled, capped, and crimped, autoclave the medium at 121°C. Following autoclaving a precipitate may form; with periodic mixing this should go back into solution within 48 hours.

Solution A

(100X solution; use 10 ml of solution A per liter of medium)

Ammonium chloride.....	100.0 g
Magnesium chloride hexahydrate.....	100.0 g
Calcium chloride dehydrate.....	40.0 g
DI Water.....	up to 1000.0 ml

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)

Potassium dibasic phosphate trihydrate.....	200.0 g
DI Water.....	up to 1000.0 ml

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)

Resazurin.....	0.5 g
DI Water.....	up to 1000.0 ml

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

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) Solution (100 X solution)

Mercaptoethanesulfonic acid.....5.0 g
DI Water.....100 ml

Distribute into screw cap test tubes, 5–6 ml per tube and seal with rubber stoppers under N₂ gas. Autoclave at 121°C 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 Solution (100 X solution)

Sodium sulfide nonahydrate.....1.5 g
DI Water.....100 ml

Distribute into screw cap test tubes, 5–6 ml per tube, and seal with Hungate stoppers. Autoclave at 121°C 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. CAUTION: if sodium sulfide comes into contact with a strong acid, hydrogen sulfide (H₂S), a very toxic gas is liberated immediately.

Cysteine Solution (100X solution)

Cysteine.....3.0 g
DI Water.....100 ml

Distribute into screw cap test tubes, 5–6 ml per tube, and seal with Hungate stoppers. Autoclave at 121°C 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.