

**ATCC medium: 2200 AQDS medium**

Solution 1 (see below).....4.0 ml  
Solution 2 (see below).....4.0 ml  
NaHCO<sub>3</sub>.....0.8 g  
Yeast extract.....4.0 g  
Tryptone.....4.0 g  
AQDS\*.....3.3 g  
Wolfe's Vitamin Solution (see below).....0.4 ml  
Wolfe's Mineral Solution (see below).....0.48 ml  
Distilled water.....392.0 ml

\*9,10-Anthra-quinone-2,6-disulfonic acid

Combine above ingredients, except for yeast extract and tryptone. Dissolve and bring to boil under 80% N<sub>2</sub>, 10% CO<sub>2</sub>, 10% H<sub>2</sub>. Remove from heat and allow to cool. Add the yeast extract and tryptone. Adjust pH to 7.2 +/- 0.2. Dispense under gas into aluminum seal tubes, 10.0 ml per tube. Autoclave at 121C for one hour.

*Solution 1:*

NH<sub>4</sub>Cl.....1.65 g  
KCl.....1.65 g  
MgCl<sub>2</sub> . 6H<sub>2</sub>O .....1.65 g  
CaCl<sub>2</sub>.....1.65 g  
Distilled water.....50.0 ml

*Solution 2:*

KH<sub>2</sub>PO<sub>4</sub>.....1.65 g  
Distilled water.....50.0 ml

*Wolfe's Vitamin Solution:*

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Biotin.....2.0 mg  
Folic acid.....2.0 mg  
Pyridoxine hydrochloride....10.0 mg  
Thiamine . HCl.....5.0 mg  
Riboflavin.....5.0 mg  
Nicotinic acid.....5.0 mg  
Calcium D-(+)-pantothenate...5.0 mg  
Vitamin B12.....0.1 mg  
p-Aminobenzoic acid.....5.0 mg  
Thioctic acid.....5.0 mg  
Distilled water.....1.0 L

*Wolfe's Mineral Solution:*

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....	1.5 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	3.0 g
MnSO <sub>4</sub> . H <sub>2</sub> O .....	0.5 g
NaCl.....	1.0 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.1 g
CoCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.1 g
CaCl <sub>2</sub> .....	0.1 g
ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.1 g
CuSO <sub>4</sub> . 5H <sub>2</sub> O .....	0.01 g
AlK(SO <sub>4</sub> ) <sub>2</sub> . 12H <sub>2</sub> O.....	0.01 g
H <sub>3</sub> BO <sub>3</sub> .....	0.01 g
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	0.01 g
Distilled water.....	1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.