

**ATCC medium: 2179 GV medium**

Trisodium citrate.....	6.0 g
K <sub>2</sub> HPO <sub>4</sub> .....	0.35 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.23 g
NH <sub>4</sub> Cl .....	0.5 g
NaCl.....	2.5 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.5 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.25 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	2.0 mg
Trace Elements Solution SL-10 (See below).....	1.0 ml
Selenite/Tungstate Solution (See below).....	1.0 ml
Wolfe's Vitamin Solution (See below).....	1.0 ml
Yeast extract.....	1.0 g
NaHCO <sub>3</sub> .....	3.0 g
Resazurin.....	0.5 mg
L-Cysteine . HCl.....	0.3 g
Na <sub>2</sub> S . 9H <sub>2</sub> O .....	0.3 g
Distilled water.....	1.0 L

Prepare separately: MgSO<sub>4</sub>, CaCl<sub>2</sub>, trisodium citrate (substrate), cysteine, and N<sub>2</sub>S. Outgas (80% N<sub>2</sub>; 20% CO<sub>2</sub>) the solutions, seal, and autoclave at 121C for 15 minutes.

Prepare the basal medium under gas, and autoclave at 121C for 15 minutes. Add other ingredients aseptically. Adjust pH to 7.0-7.2.

*Trace Elements SL-10:*

HCl (25%).....	10.0 ml
FeCl <sub>2</sub> . 4H <sub>2</sub> O .....	1.5 g
ZnCl <sub>2</sub> .....	70.0 mg
MnCl <sub>2</sub> . 4H <sub>2</sub> O .....	100.0 mg
H <sub>3</sub> BO <sub>3</sub> .....	6.0 mg
CoCl <sub>2</sub> . 6H <sub>2</sub> O .....	190.0 mg
CuCl <sub>2</sub> . 2H <sub>2</sub> O .....	2.0 mg
NiCl <sub>2</sub> . 6H <sub>2</sub> O .....	24.0 mg
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	36.0 mg
Distilled water.....	990.0 ml

Dissolve FeCl<sub>2</sub> in the HCl, dilute with water, add and dissolve the other salts; adjust pH to 6.0 with NaOH, and fill to 1.0 L with distilled water.

*Selenite/Tungstate Solution:*

NaOH.....0.5 g  
NaSeO<sub>3</sub> . 5H<sub>2</sub>O .....3.0 mg  
Na<sub>2</sub>WO<sub>4</sub> . 2H<sub>2</sub>O.....4.0 mg  
Distilled water.....1.0 L

*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