

**ATCC medium: 2304 VEN CHI2 medium**

KH <sub>2</sub> PO <sub>4</sub> .....	0.2 g
NH <sub>4</sub> Cl .....	0.25 g
NaCl.....	20.0 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	3.0 g
KCl.....	0.50 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.15 g
Trace Element SL-10 Solution (see below).....	1.0 ml
Wolfe's Vitamin Solution (see below).....	10.0 ml
Resazurin.....	1.0 mg
NaHCO <sub>3</sub> Solution (see below) .....	50.0 ml
Quinic Acid Solution (see below).....	50.0 ml
Na <sub>2</sub> S . 9H <sub>2</sub> O Solution (see below)** .....	10.0 ml
Distilled water.....	880.0 ml

Autoclave the base medium at 121C for 15 minutes. Cool under an atmosphere of 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Filter-sterilize the solutions of NaHCO<sub>3</sub>, Trace elements, and quinic acid separately. (The Trace Element Solution may be added to the Quinic Acid Solution and filtered together for convenience.)

Add sterile solutions to the base medium.  
Adjust final pH to 7.2. Maintain and dispense under the same gas mixture used above (80% N<sub>2</sub>, 20% CO<sub>2</sub>).

*Trace Element SL-10 Solution:*

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 .....	0.1 g
H <sub>3</sub> BO <sub>3</sub> .....	6.0 mg
CoCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.19 g
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 deionized water.....	990.0 ml

Dissolve FeCl<sub>2</sub> in HCl, then dilute with water and add other salts.

*Wolfe's Vitamin Solution:*

Folic acid.....	2.0 mg
Pyridoxine hydrochloride.....	10.0 mg
Riboflavin.....	5.0 mg
Biotin.....	2.0 mg
Thiamine.....	5.0 mg
Nicotinic acid.....	5.0 mg
Pantothenic acid.....	5.0 mg
Vitamin B12.....	0.1 mg
p-Aminobenzoic acid.....	5.0 mg
Thioctic acid.....	5.0 mg
Distilled deionized water.....	1.0 L

*NaHCO<sub>3</sub> Solution:*

NaHCO <sub>3</sub> .....	2.5 g
Distilled deionized water.....	50.0 ml

Filter-sterilize.

*Quinic Acid Solution:*

Quinic acid.....1.0 g  
Distilled deionized water.....50.0 ml

Filter-sterilize.

*Sodium Sulfide Solution:*

Na<sub>2</sub>S . 9H<sub>2</sub>O .....0.36 g  
Distilled deionized water.....10.0 ml

Autoclave at 121C for 15 minutes in a tube with the cap tightened.