

**ATCC medium: 1222 ASP-2 medium**

NaCl.....	18.0 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	5.0 g
KCl.....	0.6 g
NaNO <sub>3</sub> .....	0.05 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O Solution (see below) .....	2.8 ml
K <sub>2</sub> HPO <sub>4</sub> Solution (see below) .....	0.5 ml
TRIS Solution (see below).....	4.0 ml
B12 Solution (see below).....	0.1 ml
Trace Element Solution (see below).....	10.0 ml
Vitamin Solution (see below).....	1.0 ml
Na <sub>2</sub> SiO <sub>3</sub> . 9H <sub>2</sub> O Solution (see below).....	1.5 ml
Distilled water.....	1.0 L

Add salts and solutions in the order shown. Filter-sterilize.  
Aseptically dispense in 5.0 ml aliquots to 16 X 125 mm screw-capped test tubes.

*CaCl<sub>2</sub> . 2H<sub>2</sub>O Solution:*

CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	13.0 g
Distilled water.....	100.0 ml

*K<sub>2</sub>HPO<sub>4</sub> Solution:*

K <sub>2</sub> HPO <sub>4</sub> .....	1.0 g
Distilled water.....	100.0 ml

*TRIS Solution:*

Dissolve 25.0 g TRIZMA-BASE in 65.0 ml H<sub>2</sub>O. Titrate to pH 7.6 - 7.7 with concentrated HCl. Bring to 100 ml with distilled water. Let sit overnight and recheck pH.

*B12 Solution:*

Vitamin B12.....	2.0 mg
Distilled water.....	100.0 ml

*Trace Element Solution:*

EDTA.....	3.0 g
FeCl <sub>3</sub> . 6H <sub>2</sub> O .....	384.0 mg
MnCl <sub>2</sub> . 4H <sub>2</sub> O .....	432.0 mg
CoCl <sub>2</sub> . 6H <sub>2</sub> O .....	2.0 mg
ZnCl <sub>2</sub> .....	31.5 mg
CuCl or CuCl <sub>2</sub> .....	0.25 mg
H <sub>3</sub> BO <sub>3</sub> .....	342.0 mg
Distilled water.....	1.0 L

*Vitamin Solution:*

Thiamine . HCl.....	50.0 mg
Nicotinic acid.....	10.0 mg
Calcium D-(+)-pantothenate..	10.0 mg
PABA.....	1.0 mg
Inositol.....	500.0 mg
Folic acid.....	0.2 mg
Thymine.....	300.0 mg
Biotin.....	0.1 mg
Distilled water.....	100.0 ml

*Na<sub>2</sub>SiO<sub>3</sub> . 9H<sub>2</sub>O Solution:*

Na <sub>2</sub> SiO <sub>3</sub> . 9H <sub>2</sub> O.....	10.0 g
Distilled water.....	100.0 ml