

**ATCC medium: 1779 *Clostridium litorale* medium**

ATCC Medium 1770 (see below) with NaCl reduced to 10 g/L and MgSO<sub>4</sub> . 7H<sub>2</sub>O reduced to 500 mg/L.

*ATCC Medium 1770:*

*Solution 1:*

NaCl.....	60.0 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	5.0 g
NH <sub>4</sub> Cl .....	1.0 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	25.0 mg
Trace Elements Solution SL-10 (see below).....	1.0 ml
Wolfe's Vitamin Solution (see below).....	10.0 ml
Na <sub>2</sub> SeO <sub>3</sub> . 5H <sub>2</sub> O.....	15.0 mcg
Resazurin.....	1.0 mg
Betaine.....	5.86 g
Yeast extract.....	1.0 g
L-Alanine.....	2.2 g
Distilled water.....	900.0 ml

*Solution 2:*

NaHCO <sub>3</sub> .....	5.0 g
Distilled water.....	80.0 ml

Bubble under N<sub>2</sub> for 20 minutes; add to Solution 1.

*Solution 3:*

K <sub>2</sub> HPO <sub>4</sub> .....	358.0 mg
KH <sub>2</sub> PO <sub>4</sub> .....	223.0 mg
Distilled water.....	10.0 ml

Autoclave under N<sub>2</sub>.

*Solution 4:*

Na <sub>2</sub> S . 9H <sub>2</sub> O .....	300.0 mg
Distilled water.....	10.0 ml

Autoclave under N<sub>2</sub>.

Boil and cool Solution 1 under 80% N<sub>2</sub>, 20% CO<sub>2</sub>. Add Solution 2. Adjust to pH 8.3 +/- 0.1, tube and autoclave under 80% N<sub>2</sub>, 20% CO<sub>2</sub> gas phase. Add Solutions 3 and 4 aseptically to cooled, sterile medium.

*Trace Elements Solution 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 up to 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