

**ATCC medium: 1582 AOLPHA medium for *Halomonas***

NaCl.....100.0 g  
MgCl<sub>2</sub> . 6H<sub>2</sub>O .....5.0 g  
MgSO<sub>4</sub> . 7H<sub>2</sub>O .....9.5 g  
KCl.....5.0 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....0.2 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.....0.1 g  
KNO<sub>3</sub> .....0.1 g  
Peptone.....5.0 g  
Yeast extract.....1.0 g  
Metals Solution (see below).....20.0 ml  
Phosphate Solution (see below)....20.0 ml  
Vitamin Solution (see below).....1.0 ml  
Agar.....15.0 g  
Distilled water.....1.0 L

Adjust basal medium for a final pH of 7.0 and autoclave at 121C for 15 minutes. Cool to 50C and aseptically add the metals, phosphate and vitamin solutions.

*Metals Solution:*

Nitrilotriacetic acid.....10.0 g  
MgSO<sub>4</sub> . 7H<sub>2</sub>O .....29.7 g  
CaCl<sub>2</sub> . 2H<sub>2</sub>O .....3.3 g  
Na<sub>2</sub>MoO<sub>4</sub> . 2H<sub>2</sub>O.....12.7 mg  
FeSO<sub>4</sub> . 7H<sub>2</sub>O .....99.0 mg  
Metals 44 (see below).....50.0 ml  
Distilled water to.....1.0 L

Solubilize NTA with KOH. Dissolve remaining ingredients and adjust to pH 7.2 with KOH or H<sub>2</sub>SO<sub>4</sub>. Autoclave at 121C for 15 minutes, and add aseptically to sterile basal medium.

*Phosphate Solution:*

K<sub>2</sub>HPO<sub>4</sub> .....2.5 g  
KH<sub>2</sub>PO<sub>4</sub> .....2.5 g  
Distilled water.....1.0 L

Autoclave at 121C for 15 minutes. Add aseptically to sterile basal medium.

*Vitamin Solution:*

Cyanocobalamin.....	0.1 mg
Biotin.....	2.0 mg
Calcium D-(+)-pantothenate.....	5.0 mg
Folic acid.....	2.0 mg
Nicotinamide.....	5.0 mg
Pyridoxine hydrochloride.....	10.0 mg
Riboflavin.....	5.0 mg
Thiamine . HCl.....	5.0 mg
Distilled water.....	1.0 L

Filter-sterilize and add aseptically to sterile basal medium.

*Metals "44":*

EDTA.....	0.25 g
ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	1.1 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.5 g
MnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.154 g
CuSO <sub>4</sub> . 5H <sub>2</sub> O .....	0.04 g
Co (NO <sub>3</sub> ) <sub>2</sub> . 6H <sub>2</sub> O.....	0.025 g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> . 10H <sub>2</sub> O .....	0.018 g
Distilled water.....	100.0 ml

Initially add a few drops of H<sub>2</sub>SO<sub>4</sub> to the distilled water to retard precipitation.