

**ATCC medium: 1142 Allen and Arnon medium plus nitrate**

Solution A (see below).....25.0 ml  
Solution B (see below).....6.25 ml  
KNO<sub>3</sub>.....0.253 g  
NaNO<sub>3</sub>.....0.212 g  
Purified agar (if necessary).....10.0 g  
Distilled water.....969.0 ml

Combine ingredients, with the exception of Solution B, and autoclave at 121C for 15 min. Prepare Solution B as instructed below, and aseptically add to basal medium.

*Solution A:*

4% MgSO<sub>4</sub> . 7H<sub>2</sub>O .....500.0 ml  
1.2% CaCl<sub>2</sub> . 2H<sub>2</sub>O .....500.0 ml  
3.8% NaCl.....500.0 ml  
Microelements Stock Solution (see below).....500.0 ml

*Microelements Stock Solution:*

A & A FeEDTA Solution (see below).....160.0 ml  
MnCl<sub>2</sub> . 4H<sub>2</sub>O .....360.0 mg  
MoO<sub>3</sub>.....36.0 mg  
ZnSO<sub>4</sub> . 7H<sub>2</sub>O .....44.0 mg  
CuSO<sub>4</sub> . 5H<sub>2</sub>O .....15.8 mg  
H<sub>3</sub>BO<sub>3</sub> .....572.0 mg  
NH<sub>4</sub>VO<sub>3</sub> .....4.6 mg  
CoCl<sub>2</sub> . 6H<sub>2</sub>O .....8.0 mg  
Distilled water.....1090.0 ml

*A & A FeEDTA Solution:*

1. Dissolve 5.2 g KOH in 186 ml distilled water.
  2. Add 20.4 g EDTA . 2H<sub>2</sub>O.
  3. Dissolve 13.7 g FeSO<sub>4</sub> . 7H<sub>2</sub>O in 364 ml distilled water.
  4. Mix solutions 2 and 3.
  5. Bubble Millipore-filtered air through solution until color changes. (may take minutes to hours.)
- Final pH of FeEDTA solution approximately 3.5.

*Solution B:*

K<sub>2</sub>HPO<sub>4</sub> .....28.0 g  
Distilled water.....500.0 ml

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