

**ATCC medium: 1995 *Thiobacillus caldus* medium**

*Solution A:*

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.....3.0 g  
Na<sub>2</sub>SO<sub>4</sub> . 10H<sub>2</sub>O.....3.2 g  
KCl.....0.1 g  
K<sub>2</sub>HPO<sub>4</sub> .....50.0 mg  
MgSO<sub>4</sub> . 7H<sub>2</sub>O .....0.5 g  
Distilled water.....460.0 ml

Adjust solution A to pH 1.75 with H<sub>2</sub>SO<sub>4</sub>. Autoclave at 121C for 15 minutes. Maintain above 60C (see below).

*Solution B:*

Ca(NO<sub>3</sub>)<sub>2</sub> . 4H<sub>2</sub>O.....10.0 mg  
FeCl<sub>3</sub> . 6H<sub>2</sub>O .....11.0 mg  
CuSO<sub>4</sub> . 5H<sub>2</sub>O .....0.5 mg  
H<sub>3</sub>BO<sub>3</sub> .....2.0 mg  
MnSO<sub>4</sub> . H<sub>2</sub>O .....2.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> . 2H<sub>2</sub>O.....0.8 mg  
CoCl<sub>2</sub> . 6H<sub>2</sub>O .....0.6 mg  
ZnSO<sub>4</sub> . 7H<sub>2</sub>O .....0.9 mg  
Distilled water.....10.0 ml

Filter-sterilize solution B.

*Solution C:*

Glucose.....0.45 g  
Distilled water.....10.0 ml

Filter-sterilize solution C.

*Solution D:*

Sodium tetrathionate.....0.77 g  
Distilled water.....20.0 ml

Filter-sterilize solution D.

*Solution E:*

Phytigel (Sigma P-8169).....15.0 g  
Distilled water.....500.0 ml

Autoclave solution E at 121C for 15 minutes. Maintain above 60C (see below).

Maintain solutions A and E above 60C when removed from autoclave to retard rapid gelling of Phytigel. Aseptically combine solutions A through E and dispense into appropriate vessels. Complete medium will have a final pH of 2.5.

For liquid medium, omit solution E and prepare solution A in 960 ml water. Adjust solution A to pH 2.5.

For chemolithotrophic growth, omit glucose.