

**ATCC medium: 509 *Prosthecomicrobium* and *Ancalomicrobium* medium with nicotinamide**

Prepare Medium 289 (see below) as directed with the addition of 5.0 mg of nicotinamide to the Vitamin Solution.

*ATCC Medium 289:*

Ammonium sulfate.....0.25 g  
Glucose.....0.25 g  
Vitamin Solution (see below).....10.0 ml  
Modified Hutner's Basal Salts (see below).....20.0 ml  
Na<sub>2</sub>HPO<sub>4</sub> .....0.071 g  
Distilled water to.....1.0 L

*Vitamin Solution:*

Biotin.....2.0 mg  
Folic acid.....2.0 mg  
Thiamine . HCl.....5.0 mg  
Calcium D-(+)-pantothenate...5.0 mg  
Vitamin B12.....0.1 mg  
Riboflavin.....5.0 mg  
Distilled water.....1.0 L

*Modified Hutner's Basal Salts:*

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 .....3.34 g  
Ammonium molybdate.....9.25 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

Dissolve and neutralize the nitrilotriacetic acid with KOH (7.3 g); add the other ingredients and adjust the pH to 6.6 - 6.8 before bringing the volume to 1.0 L with distilled water.

*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.