ATCC Medium: 1355 Methanosarcina acetovorans Medium

NaCl……23.4 g
$MgSO_4$……6.3 g
Yeast Extract……..1.0 g
Na_2CO_3……...5.0 g
NH_4Cl……..1.0 g
KCl……...0.8 g
$CaCl_2$ x $2H_2O$……..0.14 g
Na_2HPO_4……0.6 g
Resazurin……...1.0 mg
Cysteine HCl x H_2O……...0.25 g
Na_2S x $9H_2O$……...0.25 g
Trimethylamine HCl*……3.0 g
Trace Mineral Solution (see below)……10.0 ml
Glass DI Water……990.0 ml

Final pH of medium should be 7.2. Do not adjust pH before autoclaving. After autoclaving, check and adjust pH if necessary.

Slants contain 1% Purified agar.

*Methanol or methylamine HCl may be substituted for trimethylamine HCl at a concentrated of 50 mM.

If making agar product, melt agar in a round bottom flask with all components except sodium sulfide. The best results are obtained by autoclaving under low pressure for 5 minutes. Place medium in a water bath adjusted to 50ºC with a gas mixture of 80% N_2 and 20% CO_2 flowing thorough a headspace. If there is a large amount of precipitate, add HCl and mix thoroughly by swirling. As the precipitate goes into solution the pH will decrease. A small amount of precipitate may remain. Add sodium sulfide, dispense into tubes under 80/20 gas mixture, seal with butyl rubber stoppers and autoclave at 121ºC. A precipitate will form during autoclaving but will go back into solution as the medium cools. Gently inverting the tubes before the medium solidifies will facilitate dissolution. Broth medium is prepared in the same fashion, but a water bath is not required.

Trace Mineral Solution

Nitriloacetic Acid……..1.5 g
$MgSO_4$……..3.0 g
$MnSO_4$……..0.5 g
NaCl……...1.0 g
$FeSO_4$……0.1 g
$CaCl_2$……0.1 g

$CoCl_2$…….0.1 g
$ZnSO_4$……....0.1 g
$CuSO_4$……...0.01 g
$AlK(SO_4)_2$…….0.01 g
H_3BO_4……...0.01 g
Na_2MoO_4……..0.01 g
DI Water……1000 ml

Nitriloacetic acid is soluble in a basic solution.

****Wolfe's Mineral Solution can be substituted for trace mineral solution*****

Final Product Description

- Medium will be translucent and pale yellow/slightly pink in color.
- Recommended storage temperature: 2-8ºC.