

ATCC Medium: 2728 Modified Pfennig's Medium

Solution A

CaCl ₂ ·2H ₂ O.....	0.25 g
KH ₂ PO ₄	0.34 g
NH ₄ Cl.....	0.34 g
KCl.....	0.34 g
MgSO ₄	0.50 g
Sodium acetate.....	2.00 g
Yeast Extract.....	0.10 g
NaCl.....	20.00 g
Distilled water.....	0.90 liter

Autoclave at 121°C in a cotton-stoppered anaerobic jar.

Cool to room temperature under 100% Nitrogen gas.

Add solution B, C, and D while being mixed under a head space of 100% N₂ or 95% N₂-5% CO₂.

Adjust the pH between 8.0 and 8.5 with sterile HCl (2M) or Na₂CO₃ (2M).

Distribute medium into sterile hungate tubes, seal with rubber stoppers and crimp shut. Exchange the head space for 100% N₂ or 95% N₂ – 5% CO₂.

For agar plates add 15.0 grams agar.

Filter sterilize:

Solution B

ATCC vitamin solution (MD-VS).....	1.00 ml
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Solution C

ATCC trace elements solution (MD-TMS).....	1.00 ml
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Solution D

NaHCO ₃	5.00 g
H ₂ O.....	100.00 ml

Bubble with CO₂ and, after saturation, filter sterilize under CO₂ pressure into sterile, gas tight 100 ml screw cap bottles.