

ATCC Medium 2928: M9 + 40 ug/ml Proline + 10 ug/ml Trimethoprim

M9 salts

Na ₂ HPO ₄	12.8 g
KH ₂ PO ₄	3.0 g
NaCl.....	0.5 g
NH ₄ Cl.....	1.0 g
DI Water (Agar).....	447.8 mL
DI Water (Broth).....	947.8 mL

Dissolve M9 salts and autoclave at 121°C.

For agar media only:

Agar Solution

Agar.....	15.0 g
DI Water.....	500.0 mL

Heat to dissolve agar and autoclave at 121°C.

Once both M9 Salts and Agar Solution have cooled to 50°C, aseptically add M9 salts to agar solution.

For agar and broth medias:

Add the following filter sterilized solutions:

20 % Glucose Solution (See below).....	20.0 mL
1 M MgSO ₄ Solution (See Below).....	2.0 mL
1 M CaCl ₂ Solution (See Below).....	0.1 mL
0.5% w/v Thiamine Solution (See Below).....	0.1 mL
Proline Solution (See Below).....	20.0 mL
Trimethoprim Solution (See Below).....	10.0 mL

Dispense complete medium into appropriate vessel.

20% Glucose Solution:

Glucose.....	20.0 g
DI Water.....	100.0 mL

Filter sterilize.

1M MgSO₄ Solution:

MgSO ₄	1.20 g
DI Water.....	10.0 mL

Filter sterilize.

1M CaCl₂ Solution:

CaCl ₂	1.11 g
DI Water.....	10.0 mL

Filter sterilize.

0.5% Thiamine Solution:

Thiamine.....	0.05 g
DI Water.....	10.0 mL

Filter sterilize.

Proline Solution:

Proline.....	40.0 mg
DI Water.....	20.0 mL

Filter sterilize.

Trimethoprim Solution:

Trimethoprim.....	10.0 mg
DI Water.....	10.0 mL

Add DMSO drop-wise until solution is fully dissolved.

Filter sterilize.