

ATCC Medium 125: Thiobacillus Medium

Salt Solution

(NH ₄) ₂ SO ₄	0.2 g
MgSO ₄ x 7H ₂ O	0.5 g
CaCl ₂	0.25 g
KH ₂ PO ₄	3.0 g
FeSO ₄	5.0 mg
Tap water	1000.0 mL
Sulfur (see below)	10.0 g

Prepare salt solution without sulfur and sterilize by filtration.

To prepare sulfur, follow the steps below

For 100 mL flasks:

Place approximately **1.0 g of sulfur powder** (precipitated) into a dry flask. Loosely cover each flask with a screw cap. Place flasks into the autoclave and boil @ 100°C for 30 minutes. **Do this for 3 consecutive days to sterilize.**

For test tubes:

Weigh out approximately **0.1g per test tube** and place aluminum foil over the top of the racked tubes. Follow the same instructions for sterilization process above.

Salt solution volume to Sulfur ratio:

1.0 g of precipitated Sulfur powder per 100 mL of medium
0.1 g of precipitated Sulfur powder per 10 mL of medium

Note:

**Sulfur is insoluble and has a low melting point of 106.8°C. Therefore, it must be processed separately from the salt solution. Amount is determined by vessel size as indicated above.

**Aseptically dispense the sterile salt solution into each of the sterilized Sulfur vessels (see above) making sure to carefully pour the solution down the side of the flask. The sulfur powder should not "wet"; sulfur should be floating on top of the media, not sink to the bottom. Carefully replace cap.