**Thiobacillus Medium**

\[(NH_4)_2SO_4 \quad 0.2 \text{ g}\]
\[MgSO_4 \times 7H_2O \quad 0.5 \text{ g}\]
\[CaCl_2 \quad 0.25 \text{ g}\]
\[KH_2PO_4 \quad 3.0 \text{ g}\]
\[FeSO_4 \quad 5 \text{ mg}\]

***Sulfur (see below)  Note: Sulfur is insoluble and has a low melting point (106.8) so it must be processed separately. The amount is determined by vessel size.***

Tap water 1000 ml

---

**Step 1**

**Vessel Preparation:**

For 100ml flasks:
Place approximately 1.0g 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:**
1.0g of precipitated Sulfur powder per 100ml of medium
0.1g of precipitated Sulfur powder per 10ml of medium

---

**Step 2**

**On day 3 after completion of last boil cycle:**

Prepare the salt solution above (minus the sulfur) and sterilize by filtration. Aseptically dispense the sterile solution into each of the sterilized Sulfur vessels making sure to carefully pour the solution down the side of the flask. *The sulfur powder should not "wet"….it should float on top of the liquid.* Carefully replace cap.