Main sol. J1067

FeS solution	50.00	ml
Modified Wolfe's mineral solution	50.00	ml
Agar (BD-Difco), Noble	1.50	g

1. Top layer:

Modified Wolfe's mineral solution	1000.00	ml
Trace minerals	10.00	ml

2. After cooling of the top layer solution, add per liter the following solutions (filter-sterilized):

Trace vitamins	10.00	ml
$NaHCO_3$ (8%)	5.25	ml

3. Bottom layer:

4. Autoclave both layer solutions. Shortly after autoclaving, pipette the bottom layer into each culture vessel (typically 5 ml in 60 ml glass vials, use pipette tips that have been cut to enlarge the opening). The bottom layer is allowed to cool for at least 30 min to ensure its solidification.

5. Add the top layer solution in 5 to 6 times the volume of the bottom layer to the culture vessels containing the bottom layer (e.g., 25 to 30 ml in 60 ml glass vials with 5 ml of the bottom layer).

6. Then, cap the culture vessels with silicone rubber stoppers, and replace the gas phase with a N_2-CO_2 (4:1, v/v) gas mixture.

7. Comment: The medium can be stored in culture vessels for a certain time by capping with butyl rubber stoppers, instead of silicon rubber stoppers and keeping anoxic in the vessels. Silicone rubber stopper is O_2 permeable and creates a mircoaerophilic condition in the culture vessels.