

## 7265 ASW GRADIENT PLATE

Artificial Saltwater (ASW) per liter dH<sub>2</sub>O:

27.5 g NaCl

5.38 g MgCl<sub>2</sub>\*6H<sub>2</sub>O

6.78 g MgSO<sub>4</sub>\*7 H<sub>2</sub>O

0.72 g KCl

0.2 g NaHCO<sub>3</sub>

1.4 g CaCl<sub>2</sub>\*2H<sub>2</sub>O

1 g NH<sub>4</sub>Cl

0.05 g K<sub>2</sub>HPO<sub>4</sub>

Ferrous sulfide

- Heat 250 to 300 ml dH<sub>2</sub>O to 50°C while stirring magnetically. Simultaneously heat another 300 ml dH<sub>2</sub>O in a separate beaker; set aside.
- Measure 46.2 g FeSO<sub>4</sub>\*7H<sub>2</sub>O and 39.6 g Na<sub>2</sub>S.
- Add the FeSO<sub>4</sub> to the beaker first, followed immediately by the Na<sub>2</sub>S. Stir until all Na<sub>2</sub>S has dissolved.
- Decant into a clean 500 ml glass bottle. Rinse the first beaker with pre-warmed dH<sub>2</sub>O and fill the bottle to the neck. *It is important to ensure that the equal molar amounts of the components are maintained.* Close the bottle with a rubber stopper.
- Allow the precipitate to settle. Decant the supernatant and add new dH<sub>2</sub>O or MQH<sub>2</sub>O.
- Repeat this wash procedure five times.

This stock solution may be maintained at room temperature, out of direct sunlight, for several months. You must refill the FeS with distilled or MQ water every time you remove FeS from the bottle, or the stock will oxidize rapidly.

To make gradient plates:

Top layer: 15 ml ASW per plate, plus an extra 20 ml to test pH.

5mM NaHCO<sub>3</sub>

1 µl ATCC trace minerals: 1 ml ASW

1 µl ATCC trace vitamins: 1 ml ASW\* - add after autoclaving.

Bottom later: 4 ml ASW per plate

4 ml FeS per plate

1.2% high melt agarose

Autoclave both layers separately for 20 minutes at 121°C. Immediately after autoclaving, place the top layer in an ice bath. Allow the bottom layer to cool slightly, though not to room temperature, to avoid the separation of FeS and agarose. Pipette 8.5 ml of the bottom layer into a standard Petri dish. Allow to set a minimum of 15 minutes, but no longer than 30 to avoid excessive abiotic oxidation of the iron.

While the bottom layer is setting, remove the top layer from the ice bath, and, provided it has cooled to at least room temperature, add the trace vitamin solution. Adjust the pH to between 6.0 and 6.4 by sparging with filter-sterilized CO<sub>2</sub>. The top layer should be inoculated with two to three ml of *M. ferrooxydans* per 100 ml top layer. 16 ml of top layer is pipetted carefully over the solidified bottom layer. The plates are placed in a CampyPak jar or box, and the required number of either type of microaerobic pack (see above) added for the volume of the container before it is sealed.