

## 88c: ZESTOSPHAERA MEDIUM

(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.30	g
KH <sub>2</sub> PO <sub>4</sub>	0.28	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.25	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.07	g
FeCl <sub>3</sub> x 6 H <sub>2</sub> O	0.02	g
<b>Allen's trace element solution</b>	10.00	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	2.00	g
Yeast extract (OXOID)	1.00	g
Tryptone peptone (Bacto BD)	1.00	g
Casamino acids (Difco BD)	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except thiosulfate, yeast extract, Tryptone, Casamino acids and sulfide), sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to make it anoxic and dispense medium under same gas atmosphere into serum bottles. After autoclaving add thiosulfate, yeast extract, Tryptone, Casamino acids and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Prior to inoculation check pH and adjust to 6.0, if necessary.

### Allen's trace element solution (from medium 88)

MnCl <sub>2</sub> x 4 H <sub>2</sub> O	180.00	mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> x 10 H <sub>2</sub> O	450.00	mg
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	22.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	5.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	3.00	mg
VO <sub>2</sub> SO <sub>4</sub> x 2 H <sub>2</sub> O	3.00	mg
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	1.00	mg
Distilled water	1000.00	ml

Adjust pH of final solution to 2 with 1 N HCl.