



## 1227: ZESTOSPHAERA MEDIUM

<b>Modified Brock's salt base solution</b>	1000.0000	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	2.0000	g
NaOH	0.1600	g
Resazurin	1.0000	mg
<b>Trace mineral solution</b>	10.0000	ml
Distilled water	1000.0000	ml

1. Heat the medium to boiling while bubbling with a N<sub>2</sub>-CO<sub>2</sub> (4:1, v/v) gas mixture. After boiling, cool the medium in a water bath. Continue bubbling with the same gas mixture. While the medium is still warm, add the following:

Yeast extract (Oxoid)	1.0000	g
Tryptone	1.0000	g
Casamino acids (BD-Difco)	0.5000	g
L-Cysteine HCl x H <sub>2</sub> O	0.0625	g

2. After the medium is cooled to room temperature, adjust pH to 6.0 - 6.1. Dispense the medium into culture vessels (e.g., 10 ml in Balch type tubes) under the same gas mixture, seal with butyl rubber stoppers and autoclave.

### Modified Brock's salt base solution

(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	2.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	1.80	mg
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> x 10 H <sub>2</sub> O	4.50	mg
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.22	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	0.05	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.03	mg
VO <sub>2</sub> SO <sub>4</sub> x n H <sub>2</sub> O	0.03	mg
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.01	mg
Distilled water	1000.00	ml

### Trace mineral solution

Na <sub>2</sub> -EDTA	0.50	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.15	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.10	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g



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ZnCl <sub>2</sub>	0.10	g
AlCl <sub>3</sub> x 6 H <sub>2</sub> O	0.04	g
Na <sub>2</sub> WO <sub>2</sub> x 2 H <sub>2</sub> O	0.03	g
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	0.02	g
NiSO <sub>4</sub> x 6 H <sub>2</sub> O	0.02	g
H <sub>2</sub> SeO <sub>3</sub>	0.01	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
Distilled water	1000.00	ml