

## 1508. CALDERI HABITANS MEDIUM

Sea Salts (SIGMA)	21.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.10	g
NH <sub>4</sub> Cl	0.10	g
NaNO <sub>3</sub>	0.05	g
Yeast extract	0.05	g
Fe(III) citrate	0.01	g
Trace elements solution SL-4 (see medium 14)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	2.50	g
Na-pyruvate	1.00	g
Vitamins solution (see medium 141)	10.00	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, pyruvate, vitamins, thiosulfate and sulfide, then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add pyruvate, vitamins, thiosulfate and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Stock solutions of pyruvate, vitamins and thiosulfate should be sterilized by filtration. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.