

**1131. HYDROGENIVIRGA OKINAWENSIS MEDIUM**

Modified MJ synthetic sea water (see below)	1000.0	ml
Trace vitamins (see next page)	10.0	ml
NaNO <sub>3</sub>	1.0	g
NaHCO <sub>3</sub>	1.0	g
Sulfur (powder)	3.0	g

Mix ingredients except NaHCO<sub>3</sub> and vitamin solution, adjust pH to 7.0 with NaOH, and autoclave under a N<sub>2</sub> atmosphere. Filter-sterilize 8% NaHCO<sub>3</sub> solution and add to the medium. Distribute the medium into culture vessels (e.g., 20 ml in 120 ml serum bottles) containing sulfur under a stream of a H<sub>2</sub>-CO<sub>2</sub> (4:1, v/v) gas mixture, and seal with butyl rubber stoppers. Steam medium for 3 hr on each of 3 successive days. Prior to inoculation, add trace vitamins (sterile filtrated and stocked under a N<sub>2</sub> atmosphere) to the medium. Pressurize the inoculated bottles to 200 kPa H<sub>2</sub>-CO<sub>2</sub> (4:1, v/v).

**Modified MJ synthetic sea water:**

NaCl	30.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.50	g
NH <sub>4</sub> Cl	0.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.18	g
KCl	0.33	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	1.00	mg
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	1.00	mg
Trace element solution (see below)	10.00	ml
Distilled water	1000.00	ml

**Trace element solution:**

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x X H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</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

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

***Vitamin solution:***

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine-HCl	10.00	mg
Thiamine-HCl x 2 H <sub>2</sub> O	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
D-Ca-pantothenate	5.00	mg
Vitamin B <sub>12</sub>	0.10	mg
p-Aminobenzoic acid	5.00	mg
Lipoic acid	5.00	mg
Distilled water	1000.00	ml