

**246a. REACTIVATION WITH LIQUID MEDIUM 246**

Rehydrate and grow lyophilized cells from the ampoule in liquid medium 246. Subsequent subculturing may be carried out in liquid medium or on agar plates.

**246. SEA WATER MEDIUM**

Beef extract	10.0	g
Peptone	10.0	g
Agar if required	20.0	g
Tap water	250.0	ml
Sea water*	750.0	ml

Dissolve beef extract and peptone by heating in tap water, adjust pH to 7.8 and boil for 10 min. Readjust pH to 7.3. Add agar and autoclave at 121°C for 20 min. Cool to 50°C and add warm (50°C) sterile sea water. Liquid medium without agar should be combined when cooled to room temperature.

\*Natural sea water is stored in the dark for at least three weeks to "age". If natural sea water is not available use artificial sea water.

*Artificial sea water:*

NaCl	28.13	g
KCl	0.77	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.60	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.80	g
NaHCO <sub>3</sub>	0.11	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.50	g
Distilled water	1000.0	ml