

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 ₂ x 2 H ₂ O	1.60	g
MgCl ₂ x 6 H ₂ O	4.80	g
NaHCO ₃	0.11	g
MgSO ₄ x 7 H ₂ O	3.50	g
Distilled water	1000.0	ml