

1012a: BDELLOVIBRIO BACTERIOVORUS MEDIUM

Nutrient broth (BD Difco)	0.80	g
Casamino acids (BD Bacto)	0.50	g
Yeast extract (OXOID)	0.10	g
Agar (BD Bacto)	12.00	g
MgCl ₂ x 6 H ₂ O	0.60	g
CaCl ₂ x 2 H ₂ O	0.30	g
Distilled water	1000.00	ml

1. Preparation of double-layered agar plates:

Bottom layer: Dissolve ingredients and adjust the pH of the medium to 7.2 with NaOH. Then bring the medium to a boil to dissolve the agar and autoclave (121°C for 15 min). Dry plates at room temperature for 2 - 3 days.

Top layer: Grow prey bacterium on agar slants using medium 54. Prepare 5 ml aliquots of 1 % Bacto agar in 25 mM HEPES buffer (6.0 g/l HEPES, 0.6 g/l MgCl₂ x 6 H₂O, 0.3 g/l CaCl₂ x 2 H₂O, pH 7.2) and autoclave in test tubes. After cooling to 45°C in a water bath add 0.5 ml cell suspension of the host organism, mix, and pour the top layer. For the top layer of one plate use one 24 h old agar slant culture and wash off grown cells in 0.5 ml sterile water.

2. Inoculate the top layer of the double-layer plates the same day when prepared.