

## 1012a: BDELLOVIBRIO BACTERIOVORUS MEDIUM

Final pH: 7.2

Final volume: 1000 ml

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 <sub>2</sub> x 6 H <sub>2</sub> O	0.60	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> 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 DSMZ medium 54 or medium 1. Prepare 5 ml aliquots of 1 % Bacto agar in 25 mM HEPES buffer (6.0 g/l HEPES, 0.6 g/l MgCl<sub>2</sub> x 6 H<sub>2</sub>O, 0.3 g/l CaCl<sub>2</sub> x 2 H<sub>2</sub>O, 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.

For DSM 50701: Use as prey bacterium DSM 50906 cultured on agar slants of DSM medium 54.

For DSM 115080, DSM 116241: Use as prey bacterium DSM 6125 cultured on agar slants of DSM medium 1.