

## 169: TERETINEMA MEDIUM

<b>Solution A</b>	960.00	ml
<b>Solution B</b>	20.00	ml
<b>Solution C</b>	20.00	ml

1. Dissolve ingredients of solution A (except cysteine) and sparge medium with 100% N<sub>2</sub> gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH 7.2 with KOH, then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and solution C is autoclaved under 100% N<sub>2</sub> gas atmosphere. To complete the medium appropriate amounts of solutions B and C are added to the sterile solution A in the sequence as indicated. Final pH of the complete medium should be 7.5.

2. For solid medium add 10.00 g/l agar (BD Bacto) to solution A.

### Solution A

CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.04	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
Yeast extract (OXOID)	4.00	g
D-Glucose	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Agar, for solid medium (optional)	10.00	g
Distilled water	960.00	ml

### Solution B

NaHCO <sub>3</sub>	1.00	g
Distilled water	20.00	ml

### Solution C

K <sub>2</sub> HPO <sub>4</sub> (0.5 M)	16.04	ml
KH <sub>2</sub> PO <sub>4</sub> (0.5 M)	3.96	ml

Combine 16.04 ml of 0.5 M K<sub>2</sub>HPO<sub>4</sub> with 3.96 ml of 0.5 M KH<sub>2</sub>PO<sub>4</sub> stock solution to reach a pH of around 7.4.