

### 339: WILKINS-CHALGREN ANAEROBE BROTH

This recipe contains strain-specific modifications for *Clostridium frigidum* DSM 14204 \*

Final pH: \* 7.0 - 7.2

Final volume: 1000 ml

dehydrated Wilkins-Chalgren medium (Oxoid CM0643)	33.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl	0.30	g
D-Glucose	5.00	g
NaHCO <sub>3</sub>	2.00	g
Distilled water	1000.00	ml

1. Dissolve 33.0 g/l dehydrated Wilkins-Chalgren medium (Oxoid CM0643) in distilled water and add 0.5 ml/l Na-resazurin solution (0.1% w/v), bring to the boil and cool to room temperature while sparging with 100% N<sub>2</sub> gas. Add L-cysteine-HCL x H<sub>2</sub>O (0.3 g/l), dispense the medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving check pH and adjust to pH 6.8 - 7.0, if necessary.

2. Note: In case resazurin is not colourless addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) can be used to completely reduce the medium.

\* Supplement medium after autoclaving with 5.0 g/l D-glucose and 2.0 g/l NaHCO<sub>3</sub> added from sterile stock solutions prepared under 100% N<sub>2</sub> or 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere, respectively. Adjust pH to 7.0 - 7.2, if necessary.