

## 339a: WILKINS-CHALGREN ANAEROBE BROTH (N<sub>2</sub>/CO<sub>2</sub>)

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
Na <sub>2</sub> CO <sub>3</sub>	1.00	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 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add L-cysteine-HCL x H<sub>2</sub>O (0.3 g/l), dispense the medium into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving supplement medium with 5.0 g/l D-glucose added from a sterile anoxic stock solution sterilized by filtration. Adjust pH of medium to 6.8 with a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere.

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.

For DSM 14428: Replace D-glucose with 5.0 g/l cellobiose prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration.

For DSM 14924, DSM 22607: Adjust pH of medium after autoclaving to 7.4 using a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere.

For DSM 15176, DSM 15567: Omit D-glucose.

For DSM 15248: Adjust pH of medium to 6.0.

For DSM 23669: Supplement medium after autoclaving with 0.2 g/l dithiothreitol (DTT) added from a filter-sterilized anoxic stock solution and adjust pH of complete medium to 7.2.

For DSM 28816: Replace D-glucose with 3.2 g/l Na<sub>2</sub>-fumarate prepared under 100% N<sub>2</sub> gas and sterilized by filtration. Adjust pH of complete medium to 7.2.