

**168. SPIROCHAETA AURANTIA MEDIUM****Solution A:**

D-Glucose	2.0	g
Yeast extract	2.0	g
Trypticase peptone (BD BBL)	5.0	g
Distilled water	1000.0	ml

Adjust pH to 7.5 with KOH.

**Solution B:**

1 M K-phosphate buffer, pH 7.0	10.0	ml
--------------------------------	------	----

Combine 6.15 ml of 1 M  $K_2HPO_4$  with 3.85 ml 1 M  $KH_2PO_4$  stock solution to reach a pH of around 7.0.

Sterilize *solutions A* and *B* separately by autoclaving at 121°C for 15 min and combine thereafter. Final pH of the complete medium 7.0 - 7.3.

For anaerobic cultivation sparge both solutions for 30 – 45 min with 100%  $N_2$  gas to make them anoxic. Distribute *solution A* in anoxic Hungate-type tubes or serum vials prior to autoclaving. Thereafter, add the appropriate amount of the sterile anoxic *solution B* to complete the medium.

For solid medium add 12.0 g/l agar (BD Bacto).