1. Dissolve ingredients (except bicarbonate) and adjust pH to 8.0, sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add bicarbonate from a sterile anoxic 3.2% (w/v) stock solution prepared under 80% N₂ and 20% CO₂ gas mixture (e.g., add 1 ml stock solution per 9 ml medium). Adjust pH of the complete medium to 7.5 - 7.8, if necessary.

2. After inoculation add sterile 100% H₂ gas to an overpressure of 0.5 bar.