ATCC medium: 2112 Artificial marine medium for purple sulfur bacteria #4

Solution 1:
$CaCl_2 \cdot 2H_2O$ .....0.31 g
NaCl.....25.2 g
Resazurin (0.025%).....4.0 ml
H_2O.....600.0 ml

Bring to boil. Allow to cool under N_2 until resazurin turns pink. Autoclave for 20 minutes at 121C. Allow to cool aseptically under sterile N_2 gas. Maintain under N_2 as further solutions are added.

Solution 2:
Wolfe's Mineral Solution (see below).....1.0 ml
H_3BO_3 (0.3%) .....0.9 ml
$NiCl_2 \cdot 6H_2O$ (0.02%) .....0.9 ml
Vitamin B_{12} (2.0%) .....0.9 ml
KH_2PO_4 .....0.3 g
$MgCl_2 \cdot 6H_2O$ .....0.3 g
KCl.....0.3 g
NH_4Cl .....0.3 g
H_2O.....26.3 ml

Autoclave at 121C for 15 minutes.

Wolfe's Mineral Solution:
Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)
Nitrilotriacetic acid.....1.5 g
$MgSO_4 \cdot 7H_2O$ .....3.0 g
$MnSO_4 \cdot H_2O$ .....0.5 g
NaCl.....1.0 g
$FeSO_4 \cdot 7H_2O$ .....0.1 g
$CoCl_2 \cdot 6H_2O$ .....0.1 g
$CaCl_2$.....0.1 g
$ZnSO_4 \cdot 7H_2O$ .....0.1 g
$CuSO_4 \cdot 5H_2O$ .....0.01 g
$AlK(SO_4)_2 \cdot 12H_2O$.....0.01 g
H_3BO_3 .....0.01 g
$Na_2MoO_4 \cdot 2H_2O$.....0.01 g
Distilled water.....1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

Solution 3:
$NaHCO_3$ .....1.35 g
H_2O .....270.0 ml

Combine Solutions 2 and 3. Sparge the combined solution with sterile CO_2 until pH reaches about 6.5. Then aseptically add to Solution 1.

Solution 4:
Na_2S . $9H_2O$ (NaOH washed) ....1.5 g
H_2O .....270.0 ml

Neutralize with H_2SO_4 to pH 7.5. Filter-sterilize 40.0 ml and add aseptically to the combined solutions.

Dispense combined solutions aseptically under gas as desired. Final pH should be 6.9 +/- 0.3.