ATCC Medium: 2741 Thiosulfate Gradient Medium

For Broth

ASW (see below)…….1 L
Wolfe's Mineral solution (MD-TMS)……..5.0 ml
NH_4Cl……...0.250 g
Resazurin 0.2% (wt/vol) aqueous …….…..0.10 ml

Adjust pH to 7.0 with 0.1 M NaOH. Mix well and autoclave at 121 C for 15 minutes. When the medium has cooled add the following anaerobic stock solutions to the growth medium:

Phosphate buffer……15.5 ml
$NaHCO_3$ ……20.0 ml
Vitamin (see below)……..5.0 ml
$Na_2S_2O_3 \cdot 5H_2O$ ……..10.0 ml
Cysteine…….20.0 ml

Let medium sit for several hours until it turns colorless and then add

Ferrous Sulfate…….25.0 ml

Dispense into serum bottles and Balch tubes, and purge headspace with 7.5% CO_2 – 92.5% N_2 for 1 hour

For Agar

ASW……..1L
Wolfe's Mineral solution (MD-TMS)……...10.0 ml
NH_4Cl.....0.250 g
Resazurin 0.2% (wt/vol) aqueous.....0.2 ml

Adjust pH to 7.0 with 0.1 N NaOH. Add Bacto agar (15 g / liter) and heat to a boil to dissolve the agar. Autoclave media in screw caped Erlenmeyer flask. When the medium has cooled to 45 C add the following anaerobic stocks to the growth medium. Do not let the medium cool so work fast. Additions listed are for 1 liter. Add stock solutions in the order listed: Vitamins 5 ml, Phosphate Buffer 1.50 ml, Cysteine 2 g/L, Thiosulfate solution 10 ml, and $NaHCO_3$ 2.67 ml.

Remove one ml of medium and test the pH, if the pH is greater than 7.2 add a few drops of 2N HCl. Recheck pH. Add single drops of HCl until the pH is between 6.8 and 7.2.

Once the pH has been adjusted add 2.5 ml Ferric Quinate solution (10) and stir. Medium can now be dispensed into sterile screw cap test tubes (16X125mm). When cooling, tilt on a slight incline to form a slant.

Solutions

Artificial Sea Water

NaCl…….6.43 g
$MgCl_3 \cdot 6H_2O$…….3.490 g
Na_2SO_4 ……2.74 g
KCl……0.465 g
$CaCl_2 \cdot 2H_2O$……..0.386 g
Distilled water……..1 L
Adjust pH to 7.0 with 0.1 M NaOH

Vitamin (100 ml solution)

Thiamin……..90 mg
Inositol……40 mg
D-,L-Ca^{2+} pantothenate 2 mg/ml.. 4.0 ml
Para amino benzoic acid 2 mg/ml..2.5 ml
Vitamin B_{12} 1 mg/ml……..5.0 ml
Pyridoxine (vit. B_6) 1 mg/ml……..4.0 ml
Niacin 1 mg/ml……..4.0 ml
Biotin 0.1 mg/ml……1.0 ml
Folic Acid 0.1 mg/ml…….0.4 ml

Na-bicarbonate solution (under CO_2)

Weigh out 0.672 gm into a large test tube seal tightly and autoclave. Dissolve in 10 ml sterile distilled water and add 2.67 ml to a liter of medium.

Phosphate Buffer (0.5 M)

K_2HPO_4 (dibasic), 17.418 g dissolved in 200 ml.
KH_2PO_4 (monobasic), 6.805 g dissolved in 100 ml

Add 150 ml of the dibasic to a 500 ml flask and then add the monobasic until pH 7.0. Dispense into serum bottles and purge with 100% nitrogen for 30 minutes.

Cysteine

Dissolve 0.2 gm of cysteine in about 3 ml of distilled water. Adjust to pH 6.9/7.0 with NaOH. Do not autoclave but filter sterilize directly into the medium.

Thiosulfate solution

25 gm $Na_2S_2O_3 \cdot 5H_2O$ to about 50 ml of distilled water, then bring the volume up to 100 ml. Filter sterilize into sterile serum bottles, stopper and crimp. Exchange the head space for 100% nitrogen.

Ferrous sulfate (1.0 mM)

$FeSO_4 \cdot 7H_2O$, 0.0695 g
Distilled water, 250.0 ml

Dissolve in 250 ml of 0.02 N HCl (413ul 12.1 N HCl brought up to 250 ml). Dispense into serum bottles (approximately 50 ml / bottle) bubble with 100% N_2, stopper and crimp closed. Purge head space with 100% Nitrogen.

Ferric Chloride

Make 10 mM ferric quinate solution. Dissolve 0.19 g of quinic acid to 100 ml distilled water. Add 0.27 gm of $FeCl_3 \cdot 6H_2O$ and stir to dissolve. Autoclave and store at room temperature. Discard when a precipitate becomes obvious.