ATCC Medium: 2695 (Keister’s Modified TYI-S-33) old # 1404

Casein Digest .......................... 20.00 g
Yeast Extract .......................... 10.00 g
Bovine Bile (Sigma B-8381) ....... 0.75 g
NaCl ..................................... 2.00 g
L-Cysteine HCl ......................... 2.00 g
Ascorbic Acid ......................... 0.20 g
K₂HPO₄ .................. .................. 1.00 g
KH₂PO₄ ................................. 0.60 g
Ferric Ammonium Citrate ........... 22.80 mg
DI Water ............................ 880 ml

1. Dissolve the ingredients in 880.0 ml of distilled water in the order indicated.
2. Adjust the pH to 7.0-7.2 with 1N NaOH.
3. Filter sterilize.
4. To prepare the complete medium, aseptically add 20 ml of Diamond's Vitamins Solution and 100 ml of heat-inactivated adult bovine serum.
5. Mix thoroughly and distribute 13 ml aliquots into 16 x 125 mm screw-capped borosilicate glass test tubes. Store at 4-8°C in the dark. Use within 7-10 d.

NOTE: Serum is heat-inactivated by exposure to 56°C for 30 minutes to inactivate proteins of the complement pathway.

Diamond’s Vitamin Solution

Solution 1: (DL-6,8-Thiocic acid [DL-α-Lipoic acid], 1mg/ml).
Dissolve 100 mg of DL-6,8-Thiocic acid (oxidized form, Sigma T1395) in 100 ml of absolute ethanol.

Solution 2: (Vitamin B₁₂, 0.4 mg/ml).
Dissolve 40 mg of vitamin B12 (Sigma V2876) in 100 ml distilled water.

Solution 3: (Tween 80, 50% w/v).
Dissolve 50 g of Tween 80 (Sigma P1754) in 100 ml absolute ethanol.

Solution 4:
alpha-tocopherol phosphate, disodium salt .......... 0.025 mg
d-biotin ................................................. 0.025 mg
Calciferol (Vitamin D₂) ............................. 0.250 mg
Calcium D-(+)-pantothenate ..................... 0.025 mg
Choline chloride .................................... 1.250 mg
Folic acid .......................................... 0.025 mg
i-Inositol ......................................... 0.125 mg
Menadione (Vitamin K₃) ......................... 0.025 mg
Niacin ............................................. 0.0625 mg
Niacinamide .................................... 0.0625 mg
p-aminobenzoic acid ......................... 0.125 mg
Pyridoxal HCl ................................... 0.0625 mg
Pyridoxine HCl ................................ 0.0625 mg
Riboflavin ...................................... 0.0625 mg
Thiamine HCl .................................. 0.025 mg
Vitamin A ...................................... 0.250 mg
Distilled water                                      1 L

To prepare Diamond’s Vitamin Solution combine the following:

Solution 1…………………0.4 ml
Solution 2…………………1.2 ml
Solution 3…………………0.4 ml
Solution 4…………………100.0 ml
Sterile distilled water……18.0 ml

Filter sterilize.