ATCC Medium: 2695 Keister’s Modified TYI-S-33

K2HPO4.............................................1.0 g
KH2PO4..........................................0.6 g
NaCl..............................................2.0 g
Casein Digest (BD Trypticase 211705)...20.0 g
Yeast Extract (EMD 1.03753.0500)......10.0 g
Glucose..........................................10.0 g
Dehydrated Bovine Bile (Sigma B8381)...0.75 g
L-Cysteine HCl (Sigma C7880)...........2.0 g
Ascorbic Acid (Baker B581-05).........0.2 g
Ferric Ammonium Citrate...............22.8 mg
Distilled water to......................880.0 ml

Adjust the pH to 7.0-7.1 with NaOH. Filter sterilize medium (do not autoclave). It is important to dissolve components in the specified order.

******Note: The above formulation is for internal Protistiology use only. For complete medium, see attached document.******
Complete ATCC MEDIUM 2695 Keister's Modified TYI-S-33

Modified TYI Broth 88.0 ml
Diamond's Vitamin Solution (see below) 2.0 ml
Heat-inactivated Bovine serum (HIBS)* 10.0 ml

**Modified TYI Broth**
- K2HPO4 1.0 g
- KH2PO4 0.6 g
- NaCl 2.0 g
- Casein Digest (BD Trypticase 211705) 20.0 g
- Yeast Extract (BD 212750 or EMD 1.03753.0500)** 10.0 g
- Glucose 10.0 g
- Dehydrated Bovine Bile (Sigma B8381) 0.75 g
- L-Cysteine HCl (Sigma C7880) 2.0 g
- Ascorbic Acid (Baker B581-05) 0.2 g
- Ferric Ammonium Citrate 22.8 mg
- Distilled water to 880.0 ml

1. To prepare Modified TYI Broth, dissolve the ingredients in 600.0 ml distilled water in the order indicated. Adjust the volume to 888.0 ml.
2. Adjust the pH to 7.0-7.1 with 1N NaOH. The osmolality should be 380 milliosmols/kg; adjust by increasing or decreasing NaCl.
3. Filter sterilize and distribute aseptically in 88.0 ml aliquots into pre-sterilized 100 ml screw-capped bottles. The medium can be stored at least 6 months at -20°C.
4. To prepare Diamond's Vitamin solution:
   A. Dissolve 100 mg of DL-6,8-Thioctic acid (oxidized form, Sigma T1395) in 100 ml of absolute ethanol to make Solution 1.
   B. Dissolve 40 mg of vitamin B12 (Sigma V2876) in 100 ml distilled water to make Solution 2.
   C. Dissolve 50 g of Tween 80 (Sigma P1754) in 100 ml absolute ethanol to make Solution 3.
   D. Dissolve the ingredients listed for Solution 4 in 900 ml distilled water in the order indicated. Adjust the volume to 1 L. Combine Solutions 1-4 in the amounts indicated. Filter sterilize. Store at -20°C.
5. To prepare the complete medium, aseptically add 2.0 ml of Diamond's Vitamin solution and 10.0 ml of HIBS to the 88.0 ml of Modified TYI Broth.
6. Mix thoroughly and distribute in 13 ml aliquots to 16 x 125 mm screw-capped borosilicate glass test tubes. Store at 5-9°C in the dark with the caps screwed on tightly. Use within 7-10 d.

**Diamond's Vitamin solution**
- Solution 1 (see below) 0.4 ml
- Solution 2 (see below) 1.2 ml
- Solution 3 (see below) 0.4 ml
- Solution 4 (see below) 100.0 ml
- Sterile distilled water 18.0 ml

**Solution 1**
- DL-6,8-Thioctic acid (DL-α-Lipoic acid), 1mg/ml

**Solution 2**
- Vitamin B12, 0.4 mg/ml

**Solution 3**
- Tween 80, 50% w/v
**Solution 4** (All components from Sigma)
- α-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 1.0 L

* Serum is heat-inactivated by exposure to 56°C for 30 minutes. This treatment will inactivate proteins of the complement pathway. Remove the serum from the refrigerator and aseptically distribute in 100 ml aliquots to sterile 125 ml screw-capped bottles. Immerse bottles in a 35°C water bath for 5 minutes. Do not directly transfer bottles from the refrigerator to 56°C. Transfer the bottles to a 56°C water bath and begin timing for 30 minutes. To avoid contamination, do not allow the level of the water in the bath to come in contact with the lip of the screw cap. It is best to leave one inch between the serum level in the bottle and the lip of the cap and to fill the water bath to a level just slightly above the level of the serum. To assure even heating of the serum, swirl the bottle(s) every ten minutes.

**Note:** Some suppliers provide serum already heat inactivated.
**Some lots of yeast extract may not support growth.**