

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
K ₂ HPO ₄	1.00	g
KH ₂ PO ₄	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-Thioctic acid [DL- α -Lipoic acid], 1mg/ml).

Dissolve 100 mg of DL-6,8-Thioctic 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.