

ATCC medium: 2692 Modified LYI Entamoeba medium

Potassium Phosphate Dibasic	1.00 g
Potassium Phosphate Monobasic	0.60 g
Sodium Chloride	2.00 g
Yeast extract (Sigma Y1625)	25.00 g
Liver digest neutralized (Oxoid LP0027)	5.00 g
Glucose	10.00 g
Cysteine	1.00 g
Ascorbic Acid	0.20 g
Ferric Ammonium Citrate (brown, 22.8 mg/mL).....	2.00 ml
1M Sodium Hydroxide (to pH 6.8)	7.50 ml
Water to 830 ml	

1. Dissolve the ingredients in 700.0 ml of distilled water in the order indicated. Adjust pH to 6.8 with 1 N NaOH. Adjust the volume to 830.0 ml with distilled water.
2. Distribute 83.0 ml aliquots in 100 ml screw-capped bottles and autoclave for 15 minutes. Cool to room temperature. The broth may be stored at -20°C for at least 6 months.
3. To prepare the complete medium, aseptically add 2.0 ml of Diamond's Vitamin Solution (see below) and 15 ml of heat inactivated adult bovine serum to 83.0 ml of LYI Broth. Store at 4-8°C in the dark. Use complete medium in 10 days or less.

Diamond's Vitamin Solution

Solution 1: (DL-6,8-Thioctic acid [DL-alpha-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 (All components from SIGMA):

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 to 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. Store at -20°C.