ATCC medium: 2155 LYI Giardia medium (filtered)
Available from ATCC as one tube, ready to use upon rehydration. Catalog no. PRA-2155.

LYI Broth (see below).....88.0 ml
Diamond's Vitamin Mixture 107 (see below).....2.0 ml
Heat-inactivated bovine serum.....10.0 ml

Add Vitamin Mixture and bovine serum aseptically to sterile broth. Dispense medium aseptically in 13-ml aliquots into 16 x 125 mm screw-cap tubes. Cap tightly and store at 4C in the dark. Use within 96 hours.

LYI Broth:
K_2HPO_4 .....1.0 g
KH_2PO_4 .....0.6 g
NaCl.....1.0 g
Yeast extract**.....25.0 g
Neutralized Liver Digest (Oxoid L27).....5.0 g
L-Cysteine . HCl.....1.0 g
Dextrose.....10.0 g
Ascorbic acid.....0.2 g
Ferric ammonium citrate.....22.8 mg
Distilled water to.....880.0 ml

Dissolve the solid ingredients in the order given in 600 ml of the distilled water. Adjust final broth volume to 880 ml with remaining distilled water. Adjust pH to 6.8 with NaOH. Filter-sterilize. Dispense aseptically in 88-ml aliquots into 100-xml screw-cap bottles. Modification of the initial specified formula: 5.0 g/L of the yeast extract has been substituted by 5.0 g/L of Neutralized Liver Digest (Oxoid L27). J. Eukaryot. Microbiol. 42:277-278. 1995.

**NOTE: Some lots of yeast extract may not work.

Diamond's Vitamin Mixture (107):
Water-soluble B Vitamins (see below).....500.0 ml
Biotin Solution (see below).....250.0 ml
Folic Acid Solution (see below).....250.0 ml
Lipid-soluble Vitamins A, D, and K (see below)...2,500.0 ml
Vitamin E Solution (see below).....250.0 ml

Sterilize final solution by passage through a 0.22 micron filter. Store the completed, clear mixture at -22C. Thaw and warm to room temperature before use.

If the mixture appears turbid, it should be discarded. Development of turbidity is an indication that an excess of NaOH has been used in the preparation of one of the stock solutions. J. Parasitol. 54: 1047-1056.

Water-soluble Vitamins:
Water Solution A (see below).....150.0 ml
Water Solution B (see below).....150.0 ml
Water Solution C (see below).....100.0 ml
Distilled water to.....100.0 ml

Combine solutions A, B, and C and bring total volume to 500 ml with distilled water.

Water Solution A:
Niacin.....62.5 mg
p-Aminobenzoic acid.....12.5 mg
Distilled water.....150.0 ml

Dissolve solid ingredients in boiling distilled water; restore final volume to 150 ml.

Water Solution B:
Niacinamide.....62.5 mg
Pyridoxine hydrochloride.....62.5 mg
Pyridoxal hydrochloride.....62.5 mg
Thiamine hydrochloride.....25.0 mg
Calcium pantothenate.....25.0 mg
i-Inositol.....125.0 mg
Choline chloride.....1250.0 mg
Distilled water to.....150.0 ml

Dissolve solid ingredients in 125 ml of the water. Bring final volume to 150 ml.

Water Solution C:
Riboflavin.....25.0 mg
0.1 N NaOH (as needed)
Distilled water to.....100.0 ml

Add riboflavin to 75 ml of the distilled water; add NaOH drop by drop until the riboflavin dissolves. Bring total volume to 100 ml with distilled water.

Biotin Solution:
Biotin.....30.0 mg
0.1 N NaOH (as needed)
Distilled water to.....300.0 mg

Add biotin to 200 ml of distilled water; add NaOH drop by drop until the biotin dissolves. Bring total volume to 300 ml with distilled water.

Folic Acid Solution:
Folic acid.....30.0 mg
0.1 N NaOH (as needed)
Distilled water to.....300.0 ml

Add folic acid to 200 ml of distilled water; add NaOH drop by drop until the folic acid dissolves. Bring total volume to 300 ml with distilled water.

Lipid-soluble Vitamins A, D, and K:
Lipid Solution A (see below)
Lipid Solution B (see below)
Distilled water to.....3000.0 ml

Combine Lipid Solution B with Lipid Solution A. Add distilled water to bring volume to 3000 ml.

Lipid Solution A:
Vitamin D2 (caciferol).....300.0 mg
Ethyl alcohol 9.5% (v/v).....63.0 ml
Vitamin A (crystalline alcohol)..300.0 mg

Dissolve vitamin D2 in ethyl alcohol; add vitamin A.

Lipid Solution B:
Vitamin K (menadione sodium bisulfite).....60.0 mg
Tween 80 aqueous solution 5% (v/v).....300.0 ml

Dissolve vitamin K in the Tween 80 solution.

Lipid Solution C:
Vitamin E Solution:
Vitamin E (alpha tocopherol acetate).....25.0 mg
Distilled water.....250.0 ml

Dissolve vitamin E in distilled water.