

APPENDIX: KEISTER'S MODIFIED TYI-S-SS MEDIUM

1. Prepare the Keister's Modified TYI-S-SS medium (see formulation below) by dissolving the components in 880 mL of distilled water in the order indicated, adjust the pH to 7.0 to 7.2 with 1 N NaOH, and sterile filter.

Keister's Modified TYI-S-33 Medium

Casein Digest (BD Trypticase 211705)	20.0 g
Yeast Extract (BD 212750)	10.0 g
Bovine Bile (Sigma B8381)	0.75 g
NaCl	2.0 g
L-Cysteine HCl	2.0 g
K ₂ HPO ₄	1.0 g
KH ₂ PO ₄	0.6 g
Ferric Ammonium Citrate	22.8 mg
Distilled water	880 mL

2. Prepare each of the four individual Diamond's Vitamin Stock Solutions (listed below) and sterile filter each one using a 0.22 µm filter.

Diamond's Vitamin Stock Solution 1: (DL-6,8-Thioctic acid [DL-α-Lipoic acid], 1 milligrams per milliliter)

Dissolve 100 mg of DL-6,8-Thioctic acid (oxidized form, Sigma T1395) in 100 mL of absolute ethanol.

Diamond's Vitamin Stock Solution 2: (Vitamin B₁₂, 0.4 milligrams per milliliter)

Dissolve 40 mg of vitamin B₁₂ (Sigma V2876) in 100 mL distilled water.

Diamond's Vitamin Stock Solution 3: (Tween 80, 50% w/v)

Dissolve 50 g of Tween 80 (Sigma P1754) in 100 mL absolute ethanol.

Diamond's Vitamin Stock Solution 4:

α-tocopherol phosphate, disodium salt	0.025 mg
d-biotin	0.025 mg
Calciferol (Vitamin D ₂)	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 K ₃)	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

3. Prepare Diamond's Vitamin Solution by combining the stock solutions as follows:

Diamond's Vitamin Stock Solution 1	0.4 mL
Diamond's Vitamin Stock Solution 2	1.2 mL
Diamond's Vitamin Stock Solution 3	0.4 mL
Diamond's Vitamin Stock Solution 4	100.0 mL
Sterile, distilled water	18.0 mL

4. Aseptically prepare the complete growth medium by adding 20 mL of Diamond's Vitamin Solution and 100 mL of heat-inactivated adult bovine serum to the Keister's Modified TYI-S-SS medium and mix thoroughly.
5. Distribute 13 mL aliquots into 16 × 125 mm screw-capped borosilicate glass test tubes. Store at 4°C to 8°C in the dark. Use within 7 to 10 days. Long-term storage may result in the formation of precipitates and failure to support the growth of *Giardia*.

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