

APPENDIX: MODIFIED TRYPTICASE – YEAST – MALTOSE (TYM) BASAL MEDIUM

1. Prepare the Iron Solution following the recipe below and filter sterilize using a 0.2 µm filter. Store at 2°C to 8°C and discard when the solution changes from pale pink to a dark color.

Iron Solution

Ferrous ammonium sulfate [Fe(NH ₄) ₂ (SO ₄) ₂ • 6H ₂ O]	0.5 g
Sulfosalicylic acid [C ₇ H ₆ O ₆ S]	0.05 g
Distilled, deionized water	50 mL

2. Prepare the incomplete Modified TYM Basal medium by adding all of the dry components listed to the distilled water:

Incomplete Modified TYM Basal medium

Trypticase	20 g
Yeast Extract	10 g
Maltose	5 g
L-Cystine • HCl	1 g
L-Ascorbic acid	0.2 g
K ₂ HPO ₄	0.8 g
KH ₂ PO ₄	0.8 g
Distilled water	900 mL

3. Adjust pH to 6.2 with NaOH.
4. Dispense into 500 mL bottles and autoclave at 121°C for 15 minutes and allow to cool.
5. Aseptically prepare the Complete Modified TYM Basal medium following the recipe below:

Complete Modified TYM Basal medium (500 mL)

Incomplete Modified TYM Basal medium	450 mL
Heat-Inactivated Horse Serum	50 mL
Iron Solution	3.55 mL