

APPENDIX I: MEGA2 MEDIA

The following stock powders and solutions must be prepared first:

100X Dry Microsalts

NaHCO ₃	4 g
MgSO ₄ ·7H ₂ O	2 g
NaCl	8 g
CaCl ₂	0.08 g
FeSO ₄	0.04 g

10X Dry Super Mix

Tryptone peptone	100 g
Bacto Yeast Extract	50 g
Glucose	20 g
Cysteine (free base)	5 g
KH ₂ PO ₄	41 g
K ₂ HPO ₄	122 g
100X dry microsalts	1.012 g

Vitamin K solution

Dissolve 10 mg menadione (vitamin K, Sigma-M5625) in 10 mL of 100% EtOH

Hematin-Histidine solution

1. Prepare 0.2 M histidine (pH 8.0) by dissolving 2.1 g histidine-HCl monohydrate (Sigma-H7875) in 40 mL distilled H₂O. Adjust pH from 4.0 to 8.0 with 10 N NaOH or 50% NaOH. Histidine will dissolve as pH rises. Bring final volume to 50 mL with Milli-Q water.
2. Dissolve 12 mg hematin (Sigma-H3281) in 10 mL of 0.2 M histidine (pH 8.0). Filter sterilize.

MEGA2 media (1 L broth or plates)

1. In a 1 L media bottle, combine approximately 450mL H₂O and the ingredients below:

Tween 80	100 µL
10X dry super mix	17 g
Meat extract	5g
Cellobiose	0.5 g
Maltose	0.5 g
Fructose	0.5 g
Sodium Acetate	0.5 g
Sodium Sulfate	1 g
Malic Acid	0.5 g
Agar (plates only)	15 g
2. Add a stir bar and heat the solution on a hot plate for as long as it takes to dissolve. Autoclave for 25 minutes.
3. Allow the solution to cool for about 20-25 minutes. Add the following ingredients.

Vitamin K	500 µL
Hematin-Histidine Solution	500 µL
ATCC® Vitamin Supplement (ATCC® MD-VS™)	500 µL
ATCC® Trace Mineral Supplement (ATCC® MD-TMS™)	500 µL
4. Bring to 1 L with water. For broth, filter sterilized using a 0.22 µm filter flask. Media does not need to be filter sterilized prior to pouring plates. Allowed the media to equilibrate overnight in an anaerobic chamber to achieve an anaerobic state. Place at 4°C for long-term storage.