

APPENDIX: MODIFIED HMI-9 MEDIUM¹

1. Prepare the Hypoxanthine stock solution, filter-sterilize and freeze in 100 mL aliquots.

Hypoxanthine Stock Solution

Distilled water	1,000 mL
NaOH	4 g
Add Hypoxanthine	13.6 g

2. Prepare the 10X HMI-9 supplement by adding components in the order listed below, filter-sterilize and freeze in 100 mL aliquots.

10X HMI-9 Supplement

Bathocuproine Disulfonic Acid	280 mg
L-Cysteine	1820 mg
Pyruvic Acid	1100 mg
Uracil	100 mg
L-Cytosine	100 mg
2-Mercaptoethanol	140 µL
Distilled Water	1,000 mL

3. Aseptically prepare the Modified HMI-9 medium by adding the components listed below to the Iscove's Modified Dulbecco's Medium (IMDM).

IMDM (Gibco™ 12440-046)	700 mL
Heat-Inactivated Fetal Bovine Serum (FBS)	100 mL
Serum Plus™ (Sigma-Aldrich 14008C)	100 mL
Hypoxanthine stock solution	10 mL
10X HMI-9 supplement	100 mL

Reference:

1. "Trypanosome Media Preparation Protocols." Laboratory of Molecular Parasitology, The Rockefeller University, https://tryps.rockefeller.edu/trypsru2_culture_media_preparation.html.