

APPENDIX: *BABESIA IN VITRO* GROWTH MEDIUM: DMEM/F12

1. Prepare a 10 mM hypoxanthine and 1.6 mM thymidine stock solution in distilled, deionized water and filter sterilize using a 0.22 µm filter. An equivalent prepared solution may also be used. The final concentration in the *Babesia In Vitro* Growth Medium: DMEM/F12 is 200 µM hypoxanthine and 32 µM thymidine.
2. Aseptically prepare the *Babesia* Growth Medium following the recipe below, filter sterilize using a 0.22 µm filter and store at 4°C. Use prepared medium within two weeks. Adjust the complete medium pH to 7.2, if needed.

Babesia In Vitro Growth Medium: DMEM/F12

DMEM/F12 Medium with L-glutamine	75 mL
Human Serum Type A Positive	20 mL
HB 101® supplement (Irvine Scientific® T151)	1 mL
200 mM L-glutamine stock solution (ATCC® 30-2214™)	1 mL
10 mM Hypoxanthine/1.6 mM Thymidine stock solution	2 mL

Note: To prevent culture contamination, Penicillin-Streptomycin-Amphotericin B (Antibiotic/Antimycotic) Solution (ATCC® PCS-999-002™) may be added to a final concentration of 100 IU/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B. Gentamicin may also be added to a final concentration of 100 µg/mL.