

## Complete Medium for Parasite Culture

### Reagents:

*Important Note: All solutions must be filtered using a 0.22 $\mu$ m filter before being used in culture. Solutions must stay sterile, use hood!*

- 500mL Bottle RPMI Medium 1640 (w/ 25mM HEPES and L-glutamine); [Gibco]
- 10mg/mL Gentamicine; [Gibco]
- 1.36 g/L Hypoxantine; [Sigma] *Make fresh every time!*

- 1) Add 0.013g to 10mL RPMI.
- 2) Dissolve on hot-plate with gentle heat and stirring.

- 1M HEPES; [Sigma]
- 7.5% Sodium Bicarbonate; [Gibco]
- 20% Glucose; [Sigma]
- 1M NaOH; [Sigma]
- 20% Albumax; [Gibco]

- 1) Add 25g to 125mL RPMI in beaker with stir bar.
- 2) Dissolve by gentle stirring and heating @ 37°C for at least 30'. (Until solution is yellow-brown)
- 3) Filter solution with 0.22 $\mu$ m filter.
- 4) Aliquot to 15mL sterile tubes in 6.25mL volumes.
- 5) Store @ -20°C.

- Human Serum

- 1) Incubate @ 56°C 1hr.
- 2) Filter with 0.45 $\mu$ m filter, then with 0.22 $\mu$ m filter.
- 3) Aliquot to 50mL sterile tubes in 25mL volumes.
- 4) Store @ -20°C.

For Preparation of Gametocyte Culture Medium Only...

- N-Acetyl Glucosamine; [Sigma]

### Protocol:

To a 500mL bottle of RPMI Medium 1640 add...

- 1) 3mL Gentamicine (10mg/mL Stock). **Final Concentration: 0.05 mg/mL**
- 2) 6mL Hypoxantine (1.36mg/mL Fresh Stock). **Final Concentration 0.014 mg/mL**
- 3) 22.5 mL HEPES (1M Stock). **Final Concentration 38.4 mM**
- 4) 15 mL Sodium Bicarbonate (7.5% Stock). **Final Concentration 0.20%**

- 5) 6 mL Glucose (20.0% Stock). **Final Concentration 0.20%**
- 6) 2 mL NaOH (1M Stock). **Final Concentration 3.4 mM**
- 7) 25mL Human Serum
- 8) 6.25mL 20% Albumax.
  
- 9) For gametocyte medium only, add 6.48g N-Acetyl Glucosamine. **Final Concentration 50mM**. Incubate at 37°C for 1hr to dissolve N- Acetyl Glucosamine and then sterile filter bottle using 0.22µm filter.
  
- 10) Store at 4.0°C.