

MAKING MEDIA FOR MALARIA CULTURE

(10/10/02)

0.25% Albumax in RPMI 1640 media (for 1 Liter)

1. Fill a beaker with less than 1 liter of double distilled water (ddH₂O).
2. Add 1 package of RPMI 1640 (GibcoBRL) into the stirring water.
3. Add 12.5 ml of 2 M HEPES, pH 7.4.
4. Add 2 g of sodium bicarbonate (NaHCO₃) powder.
5. Take 150 ml of this media and pour it into a separate beaker. Add 1 ml of 0.1 M hypoxanthine/1 M NaOH solution (1.361g hypoxanthine in 100 ml of **1 M NaOH**) to raise the pH of the media to 7.4. While stirring, add 2.5 gm of Albumax powder.
6. Combine the Albumax solution in with the previous media and mix well.
7. Finally, add 1 ml gentamycin (from UCSF Cell Culture Facility – 50 mg/ml).
8. Check the pH of your media. It should be around pH 7.4.
9. Bring volume up to 1 L with ddH₂O.
10. Filter the media into autoclaved 500 ml bottles using a 0.22 µm sterile Millipore filtertop.
USE STERILE TECHNIQUE AND DO THIS IN THE BIOSAFETY HOOD!
11. Label the bottle well and include the date. Store at 4 °C.

NOTES:

- Media is good for only 2 weeks. When using media for culture, pre-warm in the water bath for 15 minutes.
- All solutions for making the media are stored at 4°C, except for the hypoxanthine/NaOH solution which is kept at room temperature and gentamycin which is stored at -20°C.
- This media is good for growing W2, 3D7, Dd2, and HB3 strains. For the Brazilian D6 strain, we have to add 5% Human sera and 5% high Albumax into the media.