Alkaline Phosphatase Detection by Transmission Electron Microscopy

(by Dr. Yi Tang of Northwestern University Children's Memorial Hospital)

1. Aspirate medium away.
2. Pipette a volume of stock fixative solution (0.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.4), fix cells for approximately 25 minutes in refrigerator.
3. Scrape cell layer with tissue culture scraper from wall of tissue culture flask. Spin cells down for 2 minutes at 3000rpm.
4. Wash twice with 0.1M **PB** (or 0.1mol/L Sodium Barbital buffer, PH 9.4) solution. Spin cells down for 1 minute at 3000rpm each time. **Note: Don't use PBS to wash.**
5. Use pipette to aspirate liquid away.
6. Pipette a volume of **Incubation Solution**, wait for 30-60 minutes (60min is better) at room temperature
7. Use pipette to aspirate incubation solution away. Wash cell once with 0.1M PB solution (or PBS). Spin cells down for 1 minute at 3000rpm each time.
8. Use pipette to aspirate PB/PBS out.
9. Pipette 0.05mol/L cold **Lead Nitrate** Solution for 4 minutes at room temperature.
10. Use pipette to aspirate lead nitrate solution away.
11. Wash pellet three times with 0.1M PB solution (or PBS). Spin cells down for 1 minute at 3000rpm each time.
12. Add 1ml PBS, and submit for EM processing and analysis.

INCUBATION SOLUTION:

1, 0.1 mol/L beta-Glycerophosphate disodium salt pentahydrate	5ml
2, 0.1mol/L Sodium Barbital buffer, PH 9.4	20ml
3, 0.5mol/L Magnesium chloride	5ml
4, 0.2mol/L Calcium chloride	20ml

Filter all the solutions

LEAD NITRATE SOLUTION: 0.05mol/L (1.656g Lead nitrate + 100ml dd water)