Cell Synchronization by Double Thymidine Block or Nocodazole Block

**Double Thymidine Block**
For M/G1 cells in the morning follow **Blue** outline
For G1/S cells in the morning follow **Red** outline

1. Plate HeLa cells at 25 – 30 % confluency in a tissue culture dish (300,000 – 350,000 HeLa cells per 10 cm dish)
2. Add Thymidine to a final concentration of 2 mM (8:00 AM/2:00 PM)
3. Incubate cells for 18 hrs in tissue culture incubator
4. After 18 hrs, remove thymidine by washing with 1X PBS and adding fresh media (12:00 AM/9:00 AM)
5. Incubate for 9 hrs in tissue culture incubator
6. After 9 hrs, add thymidine to final concentration of 2 mM (9:00 AM/6:00 PM)
7. Incubate for 15 hours in tissue culture incubator
8. Release cells by washing with 1X PBS and adding fresh DMEM (12:00 AM/9:00 AM)
9a. Incubate cells for 10 hours until cells are in M/G1 and start collection
9b. Cells are released from G1/S into S. Start collection now.

**Thymidine-Nocodazole Block**
This will block cells in mitosis and release them into G1. However, Nocodazole is toxic, so cells will be sickly. Double thymidine block is preferred if possible.

1. Plate HeLa cells at 40% confluence
2. Add thymidine at 2 mM final concentration (6:00 PM) and incubate for 24 hrs
3. After 24 hours, Release from thymidine block by washing with 1X PBS and adding fresh DMEM (6:00 PM)
4. Incubate for 3 hrs in tissue culture incubator
5. After 3 hours, Add Nocodazole to a final concentration of 100 ng/mL for 12 hours (9:00 PM)
6. Remove Nocodazole by washing with 1X PBS and adding fresh DMEM
8. Cells will progress through G1, collect at your leisure