

Cell Staining with Thymidine Arrest Release

1. Trypsinize 100mM plate of confluent tissue culture cells and resuspend to 10 mLs. Dilute 5X and plate .5mLs cell suspension per well on microscope slides.
2. Grow OverNight, cells should be ~80-90% confluent.
3. Thymidine Arrest- Add thymidine to 2.0mM final concentration, grow for 17-20 hours. (Start at 2:00 p.m. so thymidine release is at ~10:00 a.m. the next day.)
4. Thymidine Release - Wash 2X with fresh medium.
5. Grow cells for 8-10 hours in fresh medium. (You can fix cells at 6:00p.m. and allow primary antibody to incubate overnight after permeabilization.)
6. Wash 1X with PBS
7. Fix with *****FRESH***** 4% paraformaldehyde for 10 minutes, follow cell staining protocol.