

Metaphase Chromosome Spreads

1. Add Colcemid to a final concentration of: 150 ng/mL (13,333X of 2mg/mL stock, ie 0.75 μ L per 10 mL) for 30 min – 4 hrs.
2. Collect floating and adherent cells by trypsinization. Spin cells at 180 xg for 10 min.
3. Aspirate supernatant leaving 0.2 mL of media and resuspend cells by tapping bottom of tube.
4. Add 6 mL of 75 mM KCl (prewarmed to 37 °C) dropwise for first 1 mL while gently vortexing. Incubate 16min at 37 °C (incubator).
5. Add four drops of fixative, invert, and spin down cells at 180 xg for 10 min.
6. Aspirate supernatant leaving 0.2 mL of hypotonic solution. Resuspend pellet by tapping and add 5 mL of fixative, 3:1 solution of methanol:glacial acetic acid (freshly prepared), dropwise for the first 1 mL while gently vortexing. Incubate 20 min at 4 °C. Spin down cells at 180 xg for 10 min.
7. Repeat step 6 two - three times until the cell pellet is colorless.
8. Resuspend the pellet in a small volume of fixative (<1 mL), until the cell suspension looks slightly cloudy.
9. Slides should be prechilled in freezer before used.
10. Draw cells into a plastic transfer pipet. Hold tip 6 inches above the slide and place 2 drops onto the slide with enough room for the drops to not touch. Stand slides on slant to dry.
11. Let slides air dry in dark.