

Preparation of Metaphase Spread

Method I

1. Add colcemid (1:1000 KaryoMAX) directly to culture dish and swirl.
Incubate 30 min to 2 hours.
 - Metaphases can be prepared without colcemid. Colcemid should increase the number of metaphase chromosomes but longer incubation times will result in shorter, more compact chromosomes.
2. Trypsinize cells as normal and wash 1X 10ml PBS. At this point it is no longer necessary to be sterile.
3. Remove as much PBS as possible and gently resuspend the cells in the residual.
4. Slowly add 0.075M KCl **dropwise** to 10ml. I add 1-2 drops then invert the tube. As soon as there are about 3 ml of KCl in the tube addition can become faster.
5. Incubate at 37°C (in a water bath) for EXACTLY 6 minutes.
6. Centrifuge at 900rpm for 5 minutes.
7. Remove as much KCl as possible and gently resuspend the cells in the residual.
8. SLOWLY add 5 ml of fixative (3:1 Methanol/Acetic acid; prepared fresh) **dropwise** and carefully mix the whole time. Adding fixative too quickly will result in clumping.
9. Centrifuge at 900rpm for 5 minutes and remove fixative
10. Slowly add 2 ml fixative **dropwise**.
11. Centrifuge 900rpm 5 minutes and remove all but 200-500µl of the fixative.
Cells are stable for extended times in fixative. If desired, store at 4°C.
12. Drop a few drops from about 18 inches high onto angled, humidified microscope slide.
13. IMMEDIATELY blow on the slide very gently.
14. Air dry at least 10 minutes. Slides are now stable for a long time.

Preparation of Metaphase Spread

Method II

1. Add colcemid (1:1000 KaryoMAX) directly to culture dish and swirl.
Incubate 30 min to 2 hours.
 - Metaphases can be prepared without colcemid. Colcemid should increase the number of metaphase chromosomes but longer incubation times will result in shorter, more compact chromosomes.
2. Trypsinize cells as normal and wash 1X 10ml PBS. At this point it is no longer necessary to be sterile.
3. Remove as much PBS as possible and gently resuspend the cells in the residual.
4. Slowly add KCl **dropwise** to 10ml. I add 1-2 drops then invert the tube. As soon as there are about 3 ml of KCl in the tube addition can become faster.
 - use 0.4% KCl for MEFs
 - use 0.57% KCl for lymphocytes, ES cells
5. Incubate at 37°C (in a water bath) for EXACTLY 8 minutes.
6. Centrifuge at 900rpm for 5 minutes.
7. Remove as much KCl as possible
8. Gently add 10 ml of cold fixative (3:1 methanol/acetic acid prepared fresh) and gently but quickly resuspend the pellet by pipetting up and down.
9. Centrifuge at 900rpm for 5 minutes
10. Repeat steps 8 and 9
11. Remove all but 1-2 ml of fixative and gently resuspend pellet in remainder
12. Store at 4°C or proceed to dropping metaphases onto slides.

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Method III

(cultured lymphocytes cells)

1. Add KaryoMax colcemid to culture at a 1:200 dilution. Incubate 3.5-4.5 hours.
2. Spin cells down in tabletop clinical centrifuge for 5 min at 1000rpm in 15ml conical tubes.
3. Remove supernatant and gently resuspend in 1ml PBS pH 7.4 by pipetting up and down with a P1000.
4. Spin cells down in microfuge for 2min at 1000 ***RCF***.
5. Very gently remove most of the supernatant with a P1000, being careful not to disturb the pellet. Gently resuspend in the residual PBS by flicking the tube or pipetting up and down with a p200.
6. Add 900µl 0.075M KCl. Incubate at 37°C for 17 min.
7. Slowly and gently add 100µl cold, fresh fixative (3:1 methanol/acetic acid). Invert to mix.
8. Spin down in a microfuge for 2min. at 1500 ***RCF***.
9. Very gently remove most of the supernatant with a P1000, being careful not to disturb the pellet. Gently resuspend in the residual fixative by flicking the tube.
10. Add 1ml cold fixative. Invert tube
11. Spin down in a microfuge for 2min. at 1500 ***RCF***
12. Very gently remove most of the supernatant with a P1000, being careful not to disturb the pellet. Gently resuspend in the residual fixative by flicking the tube.
13. Add 1ml cold fixative. Invert tube
14. Spin down in a microfuge for 2min. at 1500 ***RCF***
15. Remove all but 50-100µl of fixative, and gently resuspend cells in the remainder.
16. Store at -20°C.