Preparation of Metaphase Chromosomes from Adherent Cells

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Acetic acid, glacial
Mallinckrodt, Cat. V193
Colcemid Karyomax solution, 10 µg/ml
Invitrogen, Cat. 15210-016
ddH_2O
Methyl alcohol, anhydrous
Mallinckrodt, Cat. 3016
Phosphate buffered saline (PBS), 1X (sterile, without Ca and Mg)
Potassium chloride (KCl)
Mallinckrodt, Cat. 6858
Trypsin-EDTA
Invitrogen Corp., Cat. 25200-056

Preparations

Fixative:
Methyl alcohol/glacial acetic acid, 3:1 (volume to volume)
Prepare fresh prior to use.

Hypotonic Solution: 0.075 M (KCl)

KCl	5.6 g
Distilled water	1000 ml

Pre-warm to 37°C.

Procedure

1. Cells in flask should be 60%-80% confluent and split one day before harvesting for metaphase chromosomes.

2. Add Colcemid to flasks to a final concentration of 0.1 µg/ml (e.g., 100 µl Colcemid/10 ml medium).

3. Incubate at 37°C for 30 min to overnight (time varies depending on mitotic activity of the cells to be analyzed).

4. Remove medium and transfer it to a conical centrifuge tube.

5. Wash flask with 5-10 ml sterile PBS and keep solution in flask for about 5 min.

6. Remove contents and add to the tube containing the medium removed earlier.

7. Add 0.5–1 ml of trypsin to flask (enough to cover surface of flask).

8. Examine cells with an inverted microscope. When cells begin to lift off, immediately add 5 ml of complete medium to flask; squirt media directly onto cells which are still adherent to remove them from the flask. Pipette up and down to break up cell clumps.

9. Transfer contents to the centrifuge tube.

10. Centrifuge tube for 5 min at 1,000 rpm.

11. Remove supernatant, leaving 0.5 ml of medium in the tube and gently resuspend the pellet by flicking the tube with your fingers. Carefully add approximately 2 ml of prewarmed (37°C) 0.075 M KCl, drop-by-drop, while agitating gently. Add an additional 40-45 ml of KCl; mix well. (Note: volume of hypotonic solution is dependent upon the size of the cell pellet)

12. Incubate in 37°C water bath for 15-25 min (time will vary for different cell lines).

13. Add 4-5 drops of freshly prepared fixative to stop reaction. Centrifuge for 5 min at 1200 rpm.

14. Remove supernatant, leaving 0.5 ml of solution in the tube. Add 5 ml of freshly prepared fixative gradually, slowly down the side of the tube (or add the first 1-2 ml drop-by-drop while gently agitating the tube), and mix well by flicking tube so no clumps of cells remain. Transfer contents to a 15 ml tube. Centrifuge 5 min at 1,200 rpm.

15. Repeat step 14 twice.

17. If slides are made another day, fill the tube with freshly prepared fixative, tighten the cap, and store at 4°C.

18. Change fixative before making slides.