Metaphase Preparation from Adherent Cells

Reagents

Acetic acid, glacial
Mallinckrodt, Cat. V193
Colcemid
Boehringer Mannheim, Cat. 295-892 [10µg/ml]
Methanol
Mallinckrodt, Cat. 3016
Potassium chloride (KCl)
Mallinckrodt, Cat. 6858
Trypsin-EDTA
Gibco BRL, Cat. 25200-056

Preparations

Fixative; 3:1 metahnol – acetic acid.
Hypotonic Solution: 0.075 M potassium chloride (KCl).

Procedure

1. Cells in flask should be 60%-80% confluent and split one day before adding Colcemid.

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 4 hr (time varies depending on cells).

4. Remove medium and transfer it to a 50 ml conical tube.

5. Add 0.5–5 ml trypsin to flask (enough to cover surface of flask).

6. Examine cells with an inverted microscope. When cells begin to slough (should not take more than 5 min) immediately add 5 ml complete medium to flask and squirt media directly onto cells which are still adherent to remove them from the flask.

7. Centrifuge tube for 10 min at 1,200 rpm.

8. Remove supernatant, leaving 0.5 ml of medium in the tube and gently resuspend the pellet by flicking tube with fingers. Slowly and gently Resuspend the cells in 10 ml of potassium chloride (KCl 0.075 M).

9. Incubate in 37°C water bath for 15 min.

10. Add 3-5 drops of fresh fixative to stop reaction and trasfer to 15 ml conical tube. Centrifuge as in step 7.

11. Remove supernatant, leave 0.5 ml of potassium chloridein the tube. Add 10 ml of freshly prepared fixative (first time slowly down the side of the tube), mix carefully by pipeting up and down. Centrifuge as in step 7.

12. Repeat steps 11 twice.

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

14. Change fixative twice before making slides.