

Preparation of Human Metaphase Chromosomes

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Purpose

To culture and harvest lymphocytes to determine structural and numerical chromosomal abnormalities; to prepare chromosome preparations for CGH hybridization procedures.

Specimen

Collect 3-5 ml of heparinized whole blood (green top vacutainer tube); sodium heparin is the recommended anticoagulant.

If lithium heparin or EDTA anticoagulant (lavender top tube) has been used, process the sample with appropriate documentation to that effect. The mitotic index may be reduced. These specimens should be initiated into culture medium immediately to reduce the effect of the anticoagulant.

Reagents

Acetic acid, glacial

Antibiotic-Antimycotic 100X

(10,000 U/ml penicillin G sodium, 10,000 µg/ml streptomycin sulfate, 25 µg/ml amphotericin B)

Gibco BRL, Cat. 15240-013 (100 ml)

Colcemid, KaryoMAX Colcemid Solution (10 µg/ml)

Gibco BRL, Cat. 15210-016

Fetal Bovine Serum Qualified, heat inactivated

Gibco BRL, Cat. 16140-022 (500 ml)

L-Glutamine-200 mM, 100X

Gibco BRL, Cat. 25030-016 (100 ml)

Penicillin/Streptomycin 5,000 U/ml/5,000 µg/ml

Gibco BRL, Cat. 15070-014

Phytohaemagglutinin (PHA), HA 15

Murex Diagnostics Ltd., Dartford, England DA1 5LR

Methanol

Potassium Chloride (KCl)

Mallinckrodt, Cat. 6858

RPMI Medium 1640
Gibco BRL, Cat. 21870-050

Preparation

RPMI 1640 Full Medium Components	Amount
RPMI Medium 1640	500 ml
L-Glutamine-200 mM, 100X	5 ml
Penicillin/Streptomycin 5,000 U/ml/5,000 µg/ml	10 ml
or	
Antibiotic-Antimycotic, 100xX	5 ml
Fetal Bovine Serum Qualified, heat inactivated	100 ml

Filter sterilize full medium with 0.22 µ filter.
Medium is good for 2-3 weeks at +4°C.

Hypotonic solution
0.075 M potassium chloride (KCl) in distilled water

Fixative
Methanol/glacial acetic acid, 3:1

Procedure

Prepare mitotic cells from short-term blood cultures.

1. Add 0.7 ml of heparinized blood to 10 ml of RPMI 1640 full medium containing 0.2 ml phytohemagglutinin in T-25 flasks, and incubate for 72 hr at 37°C in a cell culture incubator. Flasks should stand upright with caps closed.
2. After 72 hr culture, add 100 µl Colcemid and mix well. Incubate for 20 min at 37°C.
3. Transfer culture to 15 ml centrifuge tubes and centrifuge at 1200 rpm for 10 min. Remove medium completely except for about 0.5 ml of supernatant remaining above the cell pellet.
4. Resuspend the cells in the remaining medium and carefully add approximately 2 ml of prewarmed (37°C) 0.075 M KCl, drop-by-drop, while agitating gently. Add an additional 8 ml of KCl, for a total of 10 ml; mix well.
5. Incubate for 15 min at 37°C in the waterbath.

6. Add a few drops of freshly prepared fixative, recap the tube, and invert to mix.
7. Centrifuge the cells and remove the supernatant as in step 3.
8. Resuspend the cells and fix the cells by adding 10 ml of fixative; the first 2 ml should be added dropwise while agitating gently.
9. After 10-15 min at room temperature, centrifuge the cells and remove the supernatant as in step 3.
10. Repeat the fixation procedure two-three more times (see notes). It is not necessary to incubate the cells between centrifugations.
11. After the last centrifugation, resuspend the cells in a small amount of fixative and drop the suspension onto a cleaned microscope slide. The quality of the metaphase spreading is dependent upon a number of factors, including humidity, air-flow, and cell concentration.

Notes

1. White blood cells in peripheral blood must be stimulated with a mitogen, inducing cell division as a prerequisite for preparation of cells in metaphase. In preparations of peripheral human blood cells, T-lymphocytes are stimulated with phytohemagglutinin.
2. Hypotonic treatment causes a swelling of the cells; the optimal time of treatment varies for different cell types and must be determined empirically.
3. Additional fixation steps are required for large or red blood cell (RBC)-contaminated pellets to get rid of debris that would interfere with slide making and banding.
4. It is advisable to make the slides the same day as the harvest. If this is not possible, fill the tube with freshly prepared fixative, tighten the cap, and store the suspension at -20°C .