2004

High Resolution Chromosomes

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

Reagents

Acetic acid, glacial
Bromodeoxyuridine (BrdU)
Sigma, Cat. B9285
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
Methotrexate (MTX)
Sigma, Cat. M8407
Methanol
Potassium chloride
Mallinckrodt, Cat. 6858
RPMI Medium 1640
Gibco BRL, Cat. 21870-050

Preparation

RPMI 1640 Full Medium

Components	**Amount**
RPMI Medium 1640	385 ml
L-Glutamine-200 mM, 100x	5 ml
Penicillin/Streptomycin 5,000 U/ml/5,000 µg/ml	10 ml
Fetal Bovine Serum Qualified, heat inactivated	100 ml

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

Fixative
Methanol/glacial acetic acid, 3:1(v/v)

MTX stock
10^{-5} M in H_2O

BrdU stock
1 mg/ml in distilled water

Procedure

1. Use T25 (with 5 ml media) or T75 flasks (with 20 ml media).

2. Initiate PHA-stimulated lymphocyte cultures. Incubate in upright position at 37°C.

3. At 72 h add from the MTX stock (10^{-5} M) to a final concentration of 10^{-7} M; mix well and incubate an additional 17 h.

4. After 17 h centrifuge the content of the flasks, remove the supernatant, and wash the pellet twice with plain media.

5. After the second wash resuspend the pellet in RPMI 1640 20% BSA and transfer to a flask.

6. Add from the BrdU stock (1 mg/ml) to a final concentration of 25 μg/ml.

7. Incubate for 5 h 30 min at 37°C.

8. During the last 10 min. of incubation add Colcemid stock (10 μg/ml) to a final concentration of 0.06 μg/ml.

9. Centrifuge cultures for 10 min.

10. Remove supernatant and add hypotonic solution, 0.075M KCl; incubate for 10 min at 37°C.

11. Add a few drops of fresh fixative and spin for 10 min.

12. Aspirate supernatant and add fixative.

13. Resuspend pellet gently in fixative and centrifuge for 5 min. Repeat this step two more times.

14. Store pellet under fixative at -20°C until ready to prepare slides.