High Resolution Human Lymphocyte Chromosomes

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

Reagents

Acetic acid, glacial
Mallinckrodt, Cat. V193
Bromodeoxyuridine (BrdU)
Sigma, Cat. B9285
Colcemid, KaryoMAX Colcemid Solution (10 μg/ml)
Invitrogen Corp., Cat.15210-016
Fetal Bovine Serum (FBS) Qualified, heat inactivated
Invitrogen Corp., Cat. 16140-022, 500 ml
L-Glutamine-200 mM, 100x
Invitrogen Corp., Cat. 25030-016
Penicillin/Streptomycin 5,000 U/ml/5,000 μg/mL
Invitrogen Corp., Cat. 15070-014
Phytohaemagglutinin (PHA), HA 15
Murex Diagnostics Ltd., Dartford, England DA1 5LR
Methotrexate (MTX)
Sigma, Cat. M8407
Methyl alcohol, anhydrous
Mallinckrodt, Cat. 3016
Potassium chloride (KCl)
Mallinckrodt, Cat. 6858
RPMI Medium 1640
Invitrogen Corp., Cat. 21870-050

Preparation

RPMI 1640 Complete 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: 0.075M KCl

KCl	5.6 g
Distilled water	1000 ml

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

MTX stock (prepare fresh)
10^{-5} M in H_2O

BrdU stock (light sensitive)
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 hr.

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

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

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

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 at 1,000 rpm.

12. Aspirate supernatant and add fixative.

13. Resuspend pellet gently in fixative and centrifuge for 5 min at 1,200 rpm. Repeat this step two more times.

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