Peripheral Blood and Bone Marrow Cell Fixation

Notes

- Use fresh fixative each time fixing cells.

Required Reagents & Equipment (Not Supplied)

Colcemid (10 µg/ml)
Hypotonic Solution – 0.075M KCl in H2O (formulated as 5.6 g potassium chloride per liter distilled water)
Fixative - 3:1 Methanol : Acetic Acid
15 ml conical tube
Centrifuge set to 1000 rpm for 5 minutes

Protocol

1. Transfer 1ml of peripheral blood, or 0.5ml of bone marrow to a 15 ml conical tube.
2. Optional (if trying to yield metaphase spreads): Add Colcemid (10 µl of 10 µg/ml for peripheral blood and 5 µl of 10 µl/ml for bone marrow) and incubate for 30 minutes prior to harvest to arrest cells at metaphase. This step gives an indication about rearrangements on hybridized probes.
3. Pellet the cells from cell suspension, leaving some liquid so pellet is not lost. Resuspend the cells in remnant liquid by tapping the tube, making sure there are no clumps.
4. Add 10 ml hypotonic solution drop by drop to the tube. Mix by inverting tube. Leave at 37°C for 20 minutes.
5. Slowly add 2 ml of fixative, mix by inverting tube.
6. Pellet the cells. Remove the supernatant, leaving some liquid to avoid loss of cells.
7. Resuspend the cells making sure there are no clumps.
8. Add 10 ml fixative making sure there are no clumps. Mix by inverting the tube gently.
9. Pellet the cells.
10. Wash 2 more times with 5 ml fixative each time.
11. Pellet and re-suspend in required volume of fixative to drop on slides. Refer to FISH Slide Dropping Protocol for dropping details.
12. Place remaining cells in fridge with 5ml of fixative for storage at ~4°C.

References

Barch MJ, Knutsen T, Spurbeck JL. The AGT Cytogenetics Laboratory Manual, Third Edition. Lippincott-Raven Philadelphia. 1991.

Empire Genomics Corp. 1000 Youngs Road Suite 207 Williamsville NY USA 14221 T 716.856.3873 F 716.431.3440 www.empiregenomics.com