

LymphoChrom

Ready to Use medium for whole blood karyotype in culture tube

Principle

Chromosomes are best studied at mitotic metaphase stage. The metaphase chromosomes are present in spontaneously dividing tissue or in cells that have been stimulated to divide in culture. The first step to get chromosome in metaphase, is tissue culturing. The techniques may differ tissue by tissue but the principle remains same, the cells should under go mitotic cell division. Proper nutrition and growth factors are an important parameter in tissue culture stage with accurate biological and physiological conditions. LymphoChrom is prepared to get optimized result. LymphoChrom is a complete medium contains that nutrition, growth factors, mitogen (PHA)and buffering agent.

Storage: -20°C

Shelf Life: 24 Months

Pack Size: 6ml

PH- 7.2

Methodology-

1. Label the provided LymphoChrom culture tube with patient name, lab ID and date of culture initiation.
2. Add 0.3-0.5ml of whole peripheral blood sample, mix it and set the culture at 37°C for 72 hrs.
3. Keep the tube in slanting position
4. After completion of 72hrs add 0.2ml Colcemid solution
5. Incubate the culture for another 30mints.
6. Centrifuge the tube at 1000rpm for 10mints.
7. Remove the supernatant and add 7-8ml hypotonic solution (0.075M KCL)
8. Incubate the tube at 37°C for 15mints
9. Add 5-6 drops of chilled fixative (3:1-Methanol: Acetic acid)
10. Centrifuge at 1000rpm for 10mints
11. Remove supernatant and add 7-8ml chilled fixative, mix it properly
12. Repeat steps 10 and 11 for 3 times
13. In the final wash, will get white lymphocyte clear pellets
14. Re-suspend the cell pellet in a small volume of chilled fixative approx. 0.4 to 0.8ml
15. Drop the cells on chilled glass slide and allow to dry
16. Metaphases can be observe under phase contrast microscope
17. Analysis will be done as per ISCN

Trouble Shooting

- † Colcemid quantity may be use as per individual lab protocol
- † Colcichine also can be used in place of Colcemid (0.2ml per tube incubation period-1hrs at 37°C)
- † Don't use the tube if medium turn turbid.
- † It is a close system culture, keep the tube screw tied at the time of incubation.
- † Incubation in normal incubator at 37°C will be preferred (No need of CO2 incubator).
- † For better results, mix the tube every 24 hrs after culture initiation

Ref

1. D.E. Rooney, Human Cytogenetics constitutional analysis, Third edition, PP. 33-44.
2. Marilyn S. Arsham, Margaret J. Barch, The AGT cytogenetics Laboratory manual, Fourth edition, PP 25-58, 87-116

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