

Instructions for chromosome analysis

Blood samples should be taken carefully (sufficient cannular diameter, reduced aspiration), as to avoid haemolysis.

1. Under appropriate (sterile) conditions, approx. 0.5 ml (25-30 drops) of heparinized venous whole blood are added and carefully mixed with 8 ml of Chromosome Medium B (or 8 ml of Chromosome medium A + 0.08 ml PHA L (cat.no. M 5030) in a tube. Cultivation time should be 72 hrs. at + 35 - +37°C.
2. 0.4 ml of Colchicin/Colcemide (10 µg/ml) (cat.no. L 6211/ L 6221) are added (corresponding to 0.48 µg/ml of medium concentration); incubate for an additional 2 hrs.
3. Cells are centrifuged for 5 min at approx. 1000 rpm. Remove supernatant and resuspend cells in 5 ml of hypotonic potassium chloride (0.075M) (cat.no. L 6413). Incubate for another 12 min at + 35 - +37°C and centrifuge the culture at 1000 rpm for 10 min.
4. Discard all but 0.25 ml of the supernatant, resuspend cells in the remaining fluid.
5. Add 5 ml of freshly prepared ice cold fixative (glacial acetic acid/methanol 1:3) dropwise to the cell suspension while gently moving the flask. Allow resting for 10 min at +2 - +8°C.
6. Centrifuge at 1000 G for 10 min, remove remaining fixative and resuspend cells in 5 ml ice-cold fixative.
7. Following another centrifugation, cells will become visible as a slightly cloudy suspension (approx. 0.5 - 1 ml). Drop 3-5 drops of cell suspension from a 30 cm distance onto a moist, non-greasy slide.
8. Air-dried slides can be stained readily using the Orcein or Giemsa staining technique.



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