Semnan BioBank

DNA EXTRACTION FROM LYMPHOCYTES

Cells should be washed and treated under the same conditions as for whole blood (see manual dna extraction from blood or lymphocytes with phenol/chloroform protocol).

Equipment and Materials

- Saline solution:
  - NaCl 3M 50 ml
  - Distilled water to 1000 ml

- SLR :
  - TRIS 2M pH 7.6 10 ml
  - MgCl2 1M 10 ml
  - NaCl 3M 6.6 ml
  - Distilled water to 2000 ml
- 50 ml SARSTEDT tubes
- Centrifuge

Procedure

The cell suspension must be treated on the day it arrives, imperatively.

1. Homogenize the culture flask with gentle manual agitation
2. Transfer the culture to a 50 ml tube
3. Centrifuge at 2000 rpm for 10 min
4. Decant the supernatant
5. Wash the pellet with 10 ml saline solution
6. Centrifuge for 5 min at 2000 rpm
7. Decant the supernatant
8. Wash the pellet with 10 ml SLR
9. Centrifuge for 5 min at 2000 rpm
10. Decant the supernatant
11. Cells may be frozen at –80°C at this stage, or be lysed for DNA extraction

DNA will be extracted from the cells using the same procedures as for cells from whole blood (see corresponding protocols: Salt Method, Phenol/Chloroform, Autogen®)