WWW.MULTIPLEXION.DE

TECHNICAL SUPPORT:
INFO@MULTIPLEXION.DE

Protocol for the extraction of genomic DNA from human and murine cell lines

Recommendation: Highly purified DNA is required for the cell line authentication service in order to work efficiently. For extraction of genomic DNA, the use of **commercial DNA extraction kits**, e.g. from Roche or Qiagen, is highly recommended. The following procedure is exemplarily adapted to the High Pure PCR Template Preparation Kit (Cat. No. 11796828001, Roche Diagnostics, Basel, Switzerland).

In order to avoid cross-contamination during DNA extraction use **aerosol-resistant filter tips** and change tips after every pipetting step! Cabinets should be used for DNA extraction in order to protect staff from being infected with hazardous material and to protect the specimens from exogeneous contaminations. Such cabinets should contain systems for filtered circulating air (equivalent to airflow within a class II biological safety cabinet), as well as UV light facilities for internal decontamination of the work area, air and pipettes.

Procedure:

1. The cell culture suspension containing 1 to 5 x 10^6 diploid cells is centrifuged in an Eppendorf tube at 2.000 x g for 4 min.

2. The supernatant is removed with a pipette using filter tips and discarded.

3. The pellet is resuspended in 200 µL PBS by vortexing. Make sure that even tiny clumps of cells are carefully resuspended.

4. Pre-warm a water bath to 70 °C.

5. 200 µL of BINDING BUFFER is added to the cell suspension and mixed by pipetting.

6. Add immediately 40 µL PROTEINASE K, mix well using a vortex and incubate at 70 °C for 10 min.

7. Add 100 µL of isopropanol (not included in the kit) to the sample, mix well and apply the whole mixture to a filter tube placed in a collection tube.

8. Centrifuge for 1 min at 8.000 x g.

9. Discard flow-through and the Collection tube, add 500 µL of INHIBITOR REMOVAL BUFFER to the upper reservoir of the Filter tube (with new Collection tube) and centrifuge again for 1 min at 8.000 x g.

10. Discard flow-through and the Collection Tube, add 500 µL of WASH BUFFER and a new Collection tube and centrifuge again for 1 min at 8.000 g and repeat this step.

11. Empty the Collection Tube and spin the Filter Tube-Collection Tube Assembly for additional 10 sec at 8.000xg.

12. Replace the Collection tube with a new 1.5 ml Eppendorf SafeLock tube and add 100 µL of ELUTION BUFFER preheated to 70 °C and centrifuge for 1 min at 8.000 x g.

13. The purified genomic DNA concentration should be adjusted to approximately 15 to 30 ng/µL

14. Store DNA at 4°C or -20°C