QUICK PROTOCOL
DNA Extraction Kit

DNA Extraction Kit
Cat. #BT06AB
Store at 15-25°C

Notes before starting

- All centrifugation steps are carried out at room temperature (15–25°C)
- Heat a water bath or heating block to 65°C
  (for animal tissues heat another one to 70°C)

Genomic DNA Extraction Protocol

1. Sample preparation
   a) Cultured Cells
   Harvesting monolayer adherent cells after cell count (do not use more than 5 x 10^6 cells)
   Aspirate the culture medium
   Wash cells using PBS (1X) then aspirate again
   Detach cells using trypsin (0.10 – 0.25%) or a cell scraper
   Centrifuge for 5 min at 300 x g. Discard the supernatant and avoid disturbing the cell pellet
   Resuspend cells in 200 µl PBS (1X)

   b) Whole Blood
   Collect the whole blood in the presence of anticoagulants such as EDTA (10 µl of 0.5M EDTA/ml Blood)

   c) Animal Tissue
   Cut ≤25 mg of animal tissue sample and transfer it to a 1.5 ml microcentrifuge tube.
   Homogenize using mechanical homogenization

2. Add 20 µl Proteinase K solution into a 1.5 ml microcentrifuge tube.
   Optional: If RNA interferes with your downstream applications, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature
3. Transfer 200 µl of the sample to the tube.
4. For animal tissue:
   a. Incubate at 65°C for 15 minutes. Mix by inversion occasionally until the cells are completely lysed.
   b. Add 200 µl of Lysis Buffer to the tube. Mix thoroughly by vortexing or pipetting.
   c. Incubate at 70°C for 10 minutes.

   For other samples:
   a. Add 200 µl of Lysis Buffer to the tube. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension (A homogeneous mixture is essential for efficient lysis).
      Note: Solution may become viscous after addition of Lysis buffer
   b. Incubate at 65°C for 15 minutes. Mix by inversion occasionally until the cells are completely lysed.
5. Add 200 µl of absolute ethanol (96–100%), Mix thoroughly by pipetting or vortexing for 15 seconds.
6. Transfer all volume (~620 µl) to the spin column, Centrifuge for 1 min at 10,000 x g. Discard flow through and the collection tube.
7. Add 500 µl of Wash Buffer I, centrifuge for 1 min at 10,000 x g.
   Discard the flow through.
8. Add 500 µl of Wash Buffer II, centrifuge for 3 min at 10,000 x g.
9. Discard the flow through.
   Optional: Repeat steps 8 – 9
10. Re-spin for 1 min. at max speed (~16,000 x g) to dry the membrane completely
11. Discard the collection tube and insert the spin column into a recovery tube.
12. Transfer 25-100 µl of elution buffer or Nuclease-Free Water directly into the spin column membrane.
13. Incubate the spin column at room temperature for 2-5 min.
14. Centrifuge for 1 min at 8,000 x g.
    Optional: Repeat elution in the same or new tube
15. Store DNA at -20°C.

For more details,
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