## GeneMATRIX Quick Blood DNA Purification Kit

## Kit for isolation of total DNA from fresh and frozen blood Cat. No. E3565 Version 1.2 January, 2008

- **Note 1:** The kit gives good results in isolation of DNA from blood, serum, plasma and other biological fluids.
- **Note 2:** Blood can be store in anticoagulants at 4°C (up to several days) or freeze at -70°C.
- **Note 3:** Once the kit is unpacked, store components at room temperature, with the exception of RNase A and Proteinase K. RNase A should be kept at 2÷8°C and Proteinase K at -20°C.
- **Note 4:** All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
- **Note 5:** The kit does not contain 96 % ethanol and PBS. To prepare sterile PBS, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na<sub>2</sub>HPO<sub>4</sub> and 0.24 g KH<sub>2</sub>PO<sub>4</sub> in 800 ml H<sub>2</sub>O. Adjust pH to 7.4 with HCl. Add H<sub>2</sub>O to 1 liter.

## **PROTOCOL**

- 1. Apply 40 μl of activation **Buffer QB** onto the spin-column (do not spin) and keep it at room temperature till transferring lysate to the spin-column.
  - Note 1: Addition of Buffer QB onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.
  - Note 2: The membrane activation should be done before starting isolation procedure.
- 2. Add to new Eppendorf tube 200 µl of liquid sample.
  - Note 1: For sample volumes less than 200  $\mu l,$  add PBS to adjust the volume to 200  $\mu l.$
- 3. Add 10 µl Proteinase K and 200 µl Sol QB buffer.

- 4. Mix thoroughly by vortexing.
- 5. Incubate for 10 min at 70° C.
- 6. Add 200 µl of **96 % ethanol**
- Mix thoroughly by vortexing
- 8. Centrifuge for 1 min at 12000 rpm.
- 9. Transfer the whole lysate to the spin-column, placed in the collection tube.
- 10. Centrifuge for 2 min at 12000 rpm.
  - Note 1: Continue centrifugation, if not all of the lysate passed through the column.
- 11. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
- 12. Add 500 μl of buffer **Wash QBX1** to spin-column and centrifuge for 1 min at 12000 rpm.
- 13. Take out spin-column, discard flow-through and place back spin-column in the collection tube.
- 14. Add 500  $\mu$ l of buffer **Wash QBX2** to spin-column and centrifuge for 2 min at 12000 rpm.
- 15. Place spin-column in a **new collection tube** (1.5-2 ml) and add 50-200 μl of **Elution buffer** (10 mM Tris-HCl, pH 8.5) heated to 70°C to elute bound DNA.
  - Note 1: Addition of eluting buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.
- 16. Incubate spin-column/collection tube assembly for 3 min at room temperature.
- 17. Centrifuge for 1 min at 12000 rpm.
- 18. Discard spin-column, cap the collection tube. Genomic DNA is ready for analysis/manipulation. It can be stored either at 2÷8° C or at -20°C.