DNAzol® PROTOCOLS FOR THE ISOLATION OF GENOMIC DNA FROM SMALL AND LARGE VOLUMES OF WHOLE BLOOD

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The following two protocols describe the use of DNAzol® (Cat. No. DN 127) for the isolation of genomic DNA from whole blood. The micro-isolation procedure is suitable for blood volumes up to 20 µl and the macro-isolation procedure is suitable for 0.5 - 5.0 ml of blood. The macro-isolation procedure includes a nuclear isolation step that reduces sample volume and protein content prior to DNA extraction. Both the micro- and macro-isolation procedures can be performed in one centrifuge tube and require approximately 20 - 30 minutes to complete. Blood samples can be fresh or stored at 4, -20 or -70 C. Samples stored frozen should thaw in the presence of the first solution used in each respective procedure.

I. MICRO-ISOLATION PROCEDURE (1 - 20 µl of whole blood)

1. Add 1 - 20 µl of whole blood to a microcentrifuge tube containing 0.5 ml of DNAzol supplemented with 2 - 5 µl of Polyacryl Carrier (Cat. No. PC 152). Immediately mix the sample by inversion and store at room temperature for 5 minutes. (Blood volumes can be increased to 50 µl, but contamination of the DNA pellet may occur. Optimal results are observed using 20 µl of whole blood.)
2. Precipitate DNA from the DNAzol - blood mixture by adding 0.25 ml of 100% ethanol. Store the sample at room temperature for 5 minutes and sediment DNA by centrifugation at 5,000 g for 5 minutes at 4 - 20 C.
3. Decant the supernatant and wash the DNA pellet once in 95% ethanol for 1 minute. Decant the ethanol wash and briefly dry the pellet at room temperature.
4. Solubilize the DNA pellet by repeated pipetting in 10 µl of distilled water, 1X TAE (40 mM Tris-acetate, 2 mM $Na_2EDTA\text{-}2H_2O$, pH 8.5) or other buffer. Solubilized DNA can be used for PCR, Southern analysis and other molecular biology applications.

II. MACRO-ISOLAION PROCEDURE

1. Isolate white cell nuclei from 0.5 - 5.0 ml of whole blood by combining 1 volume of blood with 2 volumes of cold sucrose buffer (320 mM sucrose, 1 mM Tris-HCl, 5 mM $MgCl_2$, 1% Triton X-100; pH 7.5). Mix the sample by inversion and store at room temperature for 1 minute or until the solution clears indicating hemolysis.
2. Sediment the nuclei by centrifugation at 5,000 g for 2 minutes at 4 - 20 C, and decant the supernatant. After decanting, some supernatant remains adherent to the side of the tube. Wash the tube and pellet for 10 - 20 sec with 2 volumes of lysis buffer to remove any remaining supernatant. Sediment the nuclei at 5,000 g for 2 minutes at 4 - 20 °C and decant the supernatant.
3. Disperse the nuclear pellet in DNAzol (1 ml DNAzol per 0.5 - 2.5 ml of blood) by shaking or repeated pipetting. Store the suspension for 5 minutes at room temperature.
4. Precipitate DNA by adding 0.5 volumes of 100% ethanol per 1 volume of DNAzol used to disperse the nuclear fraction. Store the sample at room temperature for 5 minutes and sediment DNA at 5,000 g for 2 minutes at 4 - 20 C. Wash the resulting DNA pellet by inversion in 95% ethanol and remove the supernatant by decanting.
5. Solubilize DNA in mM NaOH by repetitive pipetting. After complete solubilization of DNA, adjust the pH of the final product to 7.0 - 8.4 using HEPES buffer. Use the following amounts of 0.1 M or 1.0 M HEPES (free acid) per 1.0 ml of 8 mM NaOH.

Adjustment of pH in DNA samples solubilized in 8mM NaOH.
For 1 ml of 8 mM NaOH use the following amounts of 0.1 or 1M HEPES (free acid)

Final pH	0.1 M HEPES (µl)	Final pH	0.1 M HEPES (µl)	Final pH	1 M HEPES (µl)
8.4	86	7.8	117	7.2	23
8.2	93	7.5	159	7.0	32
8.0	101				

NOTES

The average expected yield is 35 µg DNA / ml of human whole blood. The amount of DNA extracted from 1 - 20 µl of human blood in the micro-isolation procedure is 35 - 700 ng. This may be below detection limits for spectrophotometric analysis. However, the DNA isolated from 1 µl of whole blood is detectable by Sybr Green® staining (Molecular Probes, Eugene, OR) during gel electrophoresis.

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