

PRODUCT: DNazol® BD

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Cat. No: DN 129

Store at room temperature. Keep tightly closed.

PRODUCT DESCRIPTION

DNazol® BD is a reagent specifically formulated for the isolation of genomic DNA from whole blood. The DNazol BD patented procedure (U. S. patent 5,945,515) is based on the use of a novel guanidine - detergent lysing solution which hydrolyzes RNA and allows the selective precipitation of DNA from the lysate. The isolation of genomic DNA from blood using DNazol BD is fast, efficient and economical. In addition to the isolation of genomic DNA, DNazol BD can also be used for the isolation of apoptotic fragments from whole blood and viral DNA from serum.

Blood samples are mixed with DNazol BD and DNA is precipitated from the resulting lysate with isopropanol. The DNA pellet is washed successively with DNazol BD and ethanol, and solubilized. The entire procedure can be completed in about 30 minutes and the isolated DNA can be used for Southern analysis, dot blot hybridization, molecular cloning, PCR and other molecular biology and biotechnology applications.

STABILITY: DNazol BD is stable at room temperature for at least two years after the date of purchase.

HANDLING PRECAUTIONS: DNazol BD contains irritants. Handle with care. Avoid contact with skin, use eye protection (shield, safety goggles). In case of contact, wash skin with a copious amount of water, seek medical attention.

PROTOCOL

Reagents required, but not supplied: isopropanol, ethanol and 8 mM NaOH.

Processing of up to 0.5 ml of blood (DNA yield 10 - 20 µg) can be performed in 2 ml microcentrifuge tubes with screw caps (cat # PP 131 and PP 132).

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| 1. LYSIS | - 1 ml DNazol BD + 0.5 ml of whole blood. |
| 2. DNA PRECIPITATION | - lysate + 0.4 ml isopropanol. Centrifuge at 6,000 g x 6 min. |
| 3. DNA WASH | - 0.5 ml DNazol BD. Centrifuge at 6,000 g x 5 min.
- 1 ml 75% ethanol. Centrifuge at 6,000 g x 5 min. |
| 4. DNA SOLUBILIZATION | - 8 mM NaOH or water. |
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The procedure is performed at room temperature. Centrifugation can be performed at 4 - 25 C.

1. LYSIS

Mix 1.0 ml of DNazol BD with 0.5 ml of whole blood. Shake vigorously by hand for 15 - 20 seconds and store at room temperature for 5 minutes. Stored blood samples have to be mixed well before taking an aliquot for the DNA isolation.

2. DNA PRECIPITATION

Add 0.4 ml of isopropanol to the DNazol BD - blood lysate. Vortex or shake vigorously and store for 5 minutes at room temperature. Sediment the precipitated DNA by centrifugation at 6,000 g for 6 minutes.

The volume of isopropanol used for the precipitation equals 0.4 volume of DNazol BD used for the lysis. Vigorous mixing of the DNazol BD - blood lysate with isopropanol dissolves protein aggregates and improves quality of the isolated DNA.

3. DNA WASH

After centrifugation, remove the supernatant and add 0.5 ml of DNazol BD to the DNA pellet. Vortex or shake the DNA pellet until it is completely dispersed. Centrifuge the resulting mixture at 6,000 g for 5 minutes. Remove the supernatant and wash the DNA pellet by mixing with 1 ml of 75% ethanol and centrifuge at 6,000 g for 5 minutes.

When using microcentrifuge tubes with snap caps, use a cotton swab to remove any residual blood accumulated in the cap and around the top of the tubes.

4. DNA SOLUBILIZATION

Decant the ethanol wash and store the tubes vertically. Remove any residual ethanol with a micropipette. Do not allow the pellet to dry. Dissolve the DNA pellet in 200 µl of 8 mM NaOH and incubate at room temperature for 3 - 5 minutes followed by repetitive pipetting or vortexing. Neutralize the alkaline DNA solution with 0.1 M HEPES (see Table). DNA can be solubilized in water, but it may take more effort to do so than with an alkaline solution. Typical yield is 20 - 40 µg of DNA / ml whole blood. Add an adequate amount of 8 mM NaOH or water to achieve a DNA concentration of about 0.1 µg/µl. At higher concentrations, the solution is extremely viscous due to the presence of high molecular weight DNA. Alkaline solutions are neutralized by CO₂ from the air. Once a month, prepare 8 mM NaOH from a 2 - 4 M NaOH stock solution that is less than 6 months old.

For 1 ml of 8mM NaOH, use the following amounts of 0.1 M or 1 M HEPES (free acid) to obtain the desired pH:

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

QUANTITATION OF DNA

Dilute an aliquot of the solubilized DNA with water, 8 mM NaOH or 1 - 3 mM Na₂HPO₄ and measure the A₂₆₀ and A₂₈₀ of the resulting solution. A slightly alkaline solution is optimal for the spectrophotometric analysis of RNA and DNA (1). Calculate the DNA content assuming that one A₂₆₀ unit equals 50 µg of double-stranded DNA/ml (2). Genomic DNA isolated with DNAzol BD has a molecular weight ranging from 40 to 100 kb and an A_{260/280} ratio of 1.7 - 1.9. The molecular weight of the isolated DNA is influenced by the extent of DNA shearing during the solubilization. In addition to genomic DNA, DNAzol BD also isolates small DNA fragments (down to 100 bp) which allows DNAzol BD to be used for the isolation of apoptotic DNA fragments and viral DNA (Note 3).

NOTES

1. Isolation of small amounts of DNA (< 10 µg) can be performed in the presence of Polyacryl Carrier. Add 3 - 5 µl of Polyacryl Carrier (cat. no. PC 152) to the initial lysate (step 1) and follow the standard protocol by precipitating the DNA and carrier mix with isopropanol.
2. The isolation procedure can be interrupted and samples can be stored at the following steps. Before or after the initial centrifugation (Step 2), the DNAzol BD lysate can be stored for at least one week at room temperature, and at least one month or one year at 4 C or -20 C, respectively. The DNA pellet can be stored in 95% ethanol for at least one week at room temperature or for one year at 4°C.
3. DNAzol BD can be used for the isolation of apoptotic DNA fragments from whole blood and viral DNA from serum. For the isolation of apoptotic fragments follow the standard protocol. For the isolation of viral DNA, substitute whole blood with an equal volume of serum and supplement the initial lysate (Step 1) with 3 - 5 µl of Polyacryl Carrier / ml of serum. Do not add more than 10 µl of Polyacryl Carrier per sample. Perform DNA precipitation using 0.5 volumes of isopropanol per one volume of DNAzol BD used for the initial lysis. Wash the DNA-carrier pellet as described in the standard protocol. Dissolve the final pellet containing viral DNA and Polyacryl Carrier in water by heating at 50°C and / or vortexing.
4. DNAzol BD can be used to isolate DNA from small quantities of whole blood (< 20 µl) or from dried blood on a blood filter card (approximately 5 µl per sample). The blood filters can be processed for DNA extraction and amplification in a single PCR tube. These protocols have been described (3) and reprints can be obtained by contacting MRC.

REFERENCES

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2. Ausubel, FM, Brent, R., Kingston, RE., Moore, DD., Seidmann, JG. and Struhl, K. (1990). *Current Protocols in Molecular Biology*, vol 3, p. A.3D.1, John Wiley & Sons, Inc. New York, NY.
3. Mackey, K., Steinkamp, A. and Chomczynski, P. (1998). DNA Extraction from Small Blood Volumes and Single-Tube DNA Extraction and Amplification Using Blood Filter Cards. *Molecular Biotechnology*, vol 9, p. 1-5.
4. Chomczynski P, Wilfinger W and Mackey K (1998) Isolation of Genomic DNA from Human, Animal, and Plant Samples with DNAzol Reagents.