GIBCO BRL

LIFE TECHNOLOGIES™

# DNAzol® BD Reagent

**WARNING: Harmful in contact with skin and if swallowed. Contact with acids liberates very toxic gas. Avoid contact with skin and eyes.**

**Cat. No.: 10974-020** **Size: 100 ml**
*CAS No.: 593-84-0* *Store at 15 to 30°C.*

This product is the subject of U.S. Patent 5,945,515.

## Description:

DNAzol® BD is a reagent specifically formulated for the isolation of genomic DNA from whole blood**.** The DNAzol BD procedure 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.

In the procedure, blood samples are mixed with DNAzol BD and DNA is precipitated from the resulting lysate with isopropanol. Next, the DNA pellet is washed successively with DNAzol BD and ethanol, and solubilized. The entire procedure can be completed in ~30 min 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 one year 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.

| | | | |
|---|---|---|---|
| 1. | Lysis | 1 ml DNAzol BD + 0.5 ml whole blood | |
| 2. | DNA Precipitation | lysate + 0.4 ml isopropanol | 6,000 × *g*, 6 min |
| 3. | DNA Wash | 0.5 ml DNAzol BD | 6,000 × *g*, 5 min |
| | | 1 ml 75% ethanol | 6,000 × *g*, 5 min |
| 4. | DNA Solubilization | 8 mM NaOH or water | |

**The procedure is carried out at room temperature. Centrifugation can be performed at 4°C to 25°C.**

1. **Lysis**
   Mix 1 ml of DNAzol BD with 0.5 ml of whole blood by vortexing or hand mixing.

2. **DNA Precipitation**
   Add 0.4 ml of isopropanol to the DNAzol BD-blood lysate. Next, vortex or shake vigorously the resulting mixture and store it for 5 min at room temperature. Sediment the precipitated DNA by centrifugation at 6,000 × *g* for 6 min.
   –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**
   Following 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 min. Next, remove supernatant and wash the DNA pellet by mixing with 1 ml of 75% ethanol and centrifuge at 6,000 × *g* for 5 min.
   **–When using microcentrifuge tubes with snap caps, be careful to remove, using a cotton swab, any residual amount of blood accumulated in the cap and around the top of the tubes.**

4. **DNA Solubilization**
   Remove the ethanol wash by decanting, store the tubes vertically, and remove the remaining ethanol wash with a micropipette. Without drying, add to the DNA pellet 200 µl of 8 mM NaOH and solubilize DNA by incubation at room temperature for 3-5 min followed by repetitive pipetting or vortexing. Neutralize the alkaline DNA solution with 0.1 M HEPES (see Table).

Alternatively to 8 mM NaOH, DNA can be solubilized in water. However, it takes more effort to fully solubilize the DNA pellet in water.
–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 solution are neutralized by $CO_2$ 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.

To adjust the DNA solution to a desired pH, add the following amounts of 0.1 M or 1 M HEPES (free acid) per 1 ml of 8 mM NaOH used for the DNA solubilization:

| Final pH | 0.1 M HEPES (µl) | Final pH | 1.0 M HEPES (µl) |
|---|---|---|---|
| 8.4 | 86 | 7.2 | 23 |
| 8.2 | 93 | 7.0 | 32 |
| 8.0 | 101 | | |
| 7.8 | 117 | | |
| 7.5 | 159 | | |

## Quantitation Of DNA And Results:

–Mix an aliquot of the solubilized DNA with 1 ml of 8 mM NaOH or 1-3 mM $Na_2HPO_4$ and measure $A_{260}$ and $A_{280}$ 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 $A_{260}$ unit equals 50 µg of double-stranded DNA/ml (2).
–Molecular weight of genomic DNA isolated with DNAzol BD ranges from 40 to 100 kb with an $A_{260}/A_{280}$ ratio of 1.7 - 1.9.
–In addition to the genomic DNA, DNAzol BD also isolates small DNA fragments (down to 100 bp). This makes it possible to use DNAzol BD for the isolation of apoptotic DNA fragments and viral DNA (see Note 3).

## Notes:

1. The isolation procedure can be interrupted and samples can be stored at the following steps:
   A) 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.
   B) The DNA pellet can be stored in 95% ethanol for a least one week at room temperature or for one year at 4°C.
2. DNAzol BD can be used for the isolation of apoptotic DNA fragments from whole blood using the standard protocol, as well as the isolation of viral DNA from serum. 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 reagent used for the initial lysis.
   Next, 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.
3. 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 (3).

## References:


1. Wilfinger, W.W., Mackey, K., and Chomczynski, P. (1997) *BioTechniques, 22*, 474 and 478.
2. Ausubel, F.M., Brent R., Kingston, R.E., Moore, D.D., Seidmann, J.G., and Struhl, K. in *Current Protocols in Molecular Biology*, vol. 3, p. 3D.1, John Wiley & Sons, Inc. New York, NY.
3. Mackey, K., Steinkamp, A., and Chomczynski, P. (1998) *Mol. Biotechnol 9*, 1.





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