7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com;
info@abpbio.com

Product Information

DNAzol BD Reagent

Catalog Number	Packaging Size
FP322	100 mL

Storage upon receipt:
- RT
- Protect from light

Product Description

DNAzol BD Reagent is a reagent specifically formulated for the isolation of genomic DNA from whole blood. The DNAzol BD Reagent 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 Reagent is fast, efficient and economical. In addition to the isolation of genomic DNA, DNAzol BD Reagent 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 Reagent and DNA is precipitated from the resulting lysate with isopropanol. The DNA pellet is washed successively with DNAzol BD Reagent and ethanol, and solubilized. The entire procedure can be completed in about 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.

Required materials not supplied

- Isopropanol
- Ethanol
- 8 mM NaOH

PROTOCOL

1. **Lysis**
   1 ml DNAzol BD Reagent + 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 reagent. 6,000 × *g*, 5 min.
   1 ml 75% ethanol. 6,000 × *g*, 5 min.
4. **DNA Solubilization**
   8 mM NaOH or water.

This procedure is carried out at room temperature. Centrifugation can be performed at 4 - 25°C.

1. Lysis

Mix 1.0 mL of DNAzol BD Reagent 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 min at room temperature. Sediment the precipitated DNA by centrifugation at 6,000 ×g for 6 min.

Note:

a. The volume of isopropanol used for the precipitation equals 0.4 volume of DNAzol BD used for the lysis.

b. 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 Reagent 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. Remove the 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, use a cotton swab to remove any residual 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 200 µL of 8 mM NaOH to the DNA pellet, and solubilize DNA by incubation at room temperature for 3-5 min followed by repetitive pipetting or vortexing. Neutralize the alkaline DNA solution with HEPES (see Table).

Note:

a. 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.

b. Weak alkaline solutions 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.

c. To adjust the DNA solution to a desired pH by the addition of HEPES. Use the following amounts of 0.1 M or 1 M HEPES (free acid) per 1 mL of 8 mM NaOH:

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

5. Quantitation of DNA and Results

Mix an aliquot of the solubilized DNA with 1 mL of 8 mM NaOH, and measure A_{260} and A_{280} of the resulting solution. Calculate the DNA content assuming that one A_{260} unit equals 50 µg of double-stranded DNA/mL. The A_{260}/A_{280} ratio of the isolated DNA is within the 1.7 - 1.9 range and with a molecular weight ranging from 40 to 100 kb.

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 2).

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.