DNA MULTIPREP™

U. S. Patent Pending | For in vitro research use | BIOTECX BULLETIN NO. 19

Genomic DNA Isolation Kit for Multiple Samples

I. INTENDED USE

This kit can be used for the isolation of pure genomic DNA from blood, mammalian cells and select tissues.

II. INTRODUCTION

Many experimental designs require the analysis of genomic DNA from a large number of samples. These include screens for gene targeting events, tests for molecular genetics, diagnosis, protocols for reverse genetics, and routine screens on transgenic mice. Although the polymerase chain reaction (PCR)* can be used for some of these purposes, Southern blot is used for many such assays because of its inherent reliability. The rapid acceptance of PCR, despite a significant false-positive-negative rate, is partly due to the disadvantages of the sample preparation for Southern blot analysis: It is labor intensive, time consuming, involves the use of hazardous chemicals and expends a good deal of reagents.

The "DNA MULTIPREP™" kit extracts genomic DNA, of the quality required for Southern blot, rapidly and from a large number of samples simultaneously. The procedure is neither labor intensive, time consuming, nor does it involve the use of hazardous organic chemicals.

III. REAGENTS SUPPLIED

CAT NO	REAGENT
BL 8611	Solution 1 Lysis Reagent
BL 8613	Solution 2 Digestion Reagent
BL 8614	Solution 3 Precipitating Reagent
BL 8616	DNA Multiprep™ Plates

All reagents should be stored at 2–8°C For added convenience reagent reservoirs are provided.

IV. ITEMS REQUIRED BUT NOT SUPPLIED

Multichannel pipette, pipette tips (50–200 µl), 50–60°C incubator, Ethanol and PBS.

V. PREPARATION OF REAGENTS

1. **Solution 1** (Lysis Reagent)

   Add entire vial contents of **Solution 2** (Digestion Reagent) into **Solution 1** Lysis Reagent; mix well.

   NOTE: This mixture should be prepared fresh just prior to use.

2. **Solution 3** (Precipitating Reagent)

   Add 1 ml of **Solution 3** to 30 ml 100% ethanol and mix well.

   NOTE: This mixture should be prepared fresh just prior to use.

VI. SAMPLE COLLECTION AND PREPARATION

A. WHITE BLOOD CELLS (WBC). Collect 1 – 2 ml of EDTA-anticoagulated blood. Count the WBCs either by automated cell counter or manual dilution with a hemocytometer. Normal WBC counts should be between 4,000 and 11,000 WBCs per microliter of whole blood. Usually, 10 million cells are more than enough to obtain approximately 50 micrograms of genomic DNA.

1) LYSING RBC'S

Centrifuge the blood sample for 5 minutes at 1000g. Discard the supernatant and add 10 ml Immulan RBC Lysing Reagent (Cat No. BL 9200), or equivalent ammonium chloride based RBC Lysing Reagent. Mix or vortex cells vigorously for 15 seconds. Incubate the cell suspension at 2–8°C for 5 minutes with occasional mixing. Add PBS to fill tube and centrifuge at 400xg, 2–8°C, for five minutes. Discard the supernatant and wash pellet with PBS. Count and suspend cells at a concentration of 1 x 10^6 cells/µl. Add 20 µl (2 x 10^6 cells) of cell suspension to each well of the Multiprep™ Plate.

2) FICOLL-HYPAQUE PERIPHERAL BLOOD LYMPHOCYTE ISOLATION METHOD

Collect 2 ml of EDTA-anticoagulated blood. Transfer to 15 ml conical tube. Dilute blood with 2.5 ml PBS. Carefully layer the diluted blood onto 2 ml of Ficoll-Hypaque. Centrifuge tube for 30 minutes at 600g. The peripheral blood lymphocytes are harvested from the plasma Ficoll-Hypaque interphase with a pasteur pipette. Wash cells 2X with PBS, count, and resuspend cells at a concentration of 1 x 10^5 cells/µl. Add 20µl (2 x 10^6 cells) of cell suspension to each well of Multiprep™ Plate.

B. CULTURED CELLS. Grow the cells in conventional bottles or plates. Remove cells with a trypsin treatment. Wash and centrifuge cells 2X with PBS. Resuspend the cells at a concentration of 1 x 10^5 cells/µl. Add 20 µl (2 x 10^6 cells) of cell suspension to each well of Multiprep™ Plate.

C. CULTURING CELLS DIRECTLY IN DNA MULTIPREP™ 96 WELL PLATES. Cells may be cultured in the DNA Multiprep™ 96 Well Plates. When the cells have reached confluence, aspirate off the cell culture supernatant. Gently wash the cells 2X with 100 µl of PBS. Proceed to Section VII Step 2.

D. TRANSGENIC MICE SCREENING. Excise a 2 sq mm tissue sample from mouse ear to be screened and add to a single well of the DNA Multiprep™ Plate. Proceed to Section VII Step 2.

NOTES:

1. Tissue sample must be completely submerged in the Lysis Reagent. If the tissue sample floats use a pipette tip to completely submerge sample.
2. To obtain maximal yield the plates should be centrifuged at 2500 rpm for 12 minutes after step VII. 4.

VII. PROCEDURE FOR GENOMIC DNA ISOLATION

1. The washed cells (20µl) are transferred into the DNA Multiprep™ Plate.
2. Add 50µl of **Solution 1** Lysis Reagent prepared as previously described in section V to each well.
3. Incubate plate at 50–60°C overnight in a humid atmosphere.
4. Add 2 volume (150µl) of cold **Solution 3** Precipitating reagent to each well. Keep plates at room temperature for 30 minutes.
5. Discard the supernatant by gently inverting the plate (DO NOT ASPIRATE).
6. Gently wash the plate 3X with 70% ethanol, discarding the ethanol during each wash cycle by inversion. (DO NOT ASPIRATE)
7. After the final wash, tilt the plate and air dry for 15 – 20 minutes. The genomic DNA is precipitated in the well and is bound to the surface of the DNA Multiprep™ Plate. The precipitated DNA is now ready for further experiments.

   The DNA pellet can be resuspended in the appropriate buffer for use as specified in subsequent procedures, such as restriction enzyme digest, Southern blot or PCR.

VIII. INTENDED USE

For subsequent procedures requiring RNA-free DNA the RNA should be destroyed by adding DNase-free pancreatic RNase to a final concentration of 50µg/ml (i.e., add 1 µl of 5 mg/ml RNase stock solution per 100µl of sample DNA.) and incubating 1 hour at room temperature.

PREPARATION OF RNase

Before use, the pancreatic RNase (RNase A) should be dissolved at a concentration of 5 mg/ml in 10mm Tris-HCl (pH 7.5), 15mm NaCl, heated to 100°C for 15 minutes and allowed to cool slowly at room temperature. The RNase A solution should then be dispensed into aliquots and stored at –20°C.

IX. EXPECTED GENOMIC DNA RECOVERY

In general the protocol gives 80% of theoretical DNA recovery. The yield is reliable and quantitative from sample to sample.

Sample	Yield
1 ml whole blood	20 – 50µg
Confluent ES clone cells	5 – 10µg
Mouse ear	5 – 10µg

** Polymerase Chain Reaction (PCR) technology is covered by U.S. Patents issued to Cetus Corporation.*

CAT NO. FOR BLOOD CELLS			CAT NO. FOR MOUSE EAR
BL-8610C	1 Plate	96 Samples	BL-8610T
BL-8620C	2 Plate	192 Samples	BL-8620T
BL-8650C	5 Plate	480 Samples	BL-8650T

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