Blood DNA Purification Kit

Catalog No.: B0008

Description

The ***EZ*Bioscience**® Blood DNA Purification Kit provides a simple, reliable, and rapid method for isolating genomic DNA from fresh or frozen anti-coagulated blood, without toxic substances such as phenol or chloroform. The purified genomic DNA is suitable for all common molecular biological applications directly: PCR, cloning, DNA sequencing, digestion with restriction endonucleases, and membrane hybridization (e.g., Southern blot analysis). Red blood cells are first eliminated by RCL buffer, and then DNA containing leukocytes are homogenized by Lysis Buffer, which contains strong denaturant substance. The lysate is then applied to the column to bind the genomic DNA. Any impurities are effectively removed by subsequent washing with Wash Buffer and 80% ethanol. Finally, the purified genomic DNA is eluted with the Elution Buffer, which can be used in a variety of downstream applications.

Components

Components	B0008 (100 Preps)
RCL Buffer	60 ml
Lysis Buffer	55 ml
Wash Buffer*	12 ml
Elution Buffer	25 ml
Spin Columns for DNA (with Collection Tubes)	100 preps

*Before using for the first time, add 48 ml of 100% ethanol to the Wash Buffer.

Storage

Store the RCL Buffer at 2 ~ 8°C upon reception. Store other components at room temperature (Divide the Elution Buffer into 3 aliquots upon reception and store at room temperature). When using these buffers, be careful to avoid of contamination.

Notice

1. When collecting blood samples, citric acid (sodium) or EDTA is recommended as the anti-coagulant, but heparin (sodium) is not recommended. Otherwise, the experiment results will be significantly affected.
2. Wash Buffer must be mixed with 48 ml ethyl alcohol absolute before it can be used.
3. Before the experiment, 120 ml 80% ethanol should be prepared with sterilized water for column washing.
4. Elution Buffer should be pre-heated to 60°C before the experiment starts.
5. DNA purification should be carried out at room temperature and not be placed on ice.

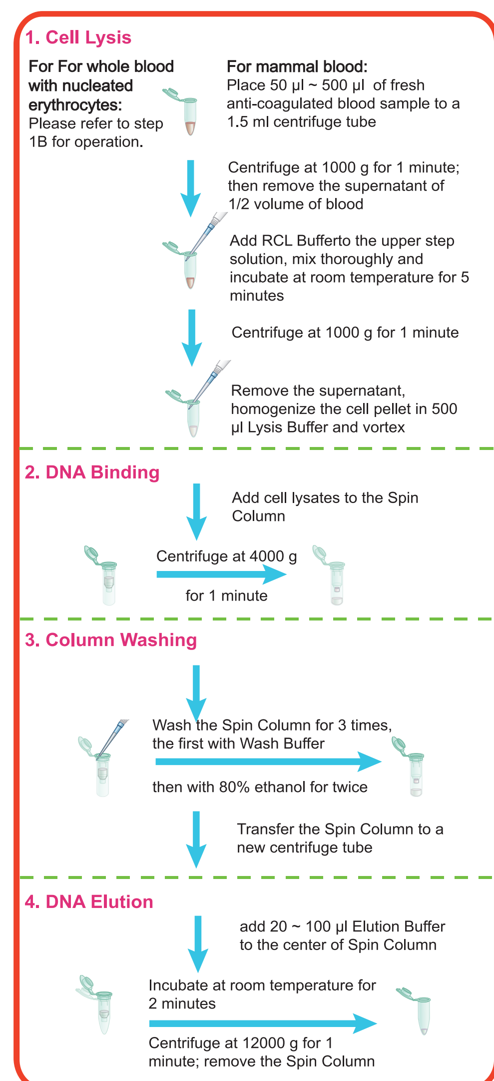
Processing Capacity and Expected Yields

Yields of genomic DNA will vary from sample to sample depending on the amount and type of material. In addition, the quality of starting material also affect DNA yields.

The following table can be used as a reference:

Blood Source	Volume	Typical Yield
Mammal	50 μl ~ 500 μl	3 ~ 6 μg/100 μl sample
Fish, amphibian, reptile or bird	5 ~ 20 μl	5 ~ 15 μg/5 μl sample

Experimental Procedure Overview:



Protocol

Sample Lysis

1A. For mammalian blood:

a1. Plasma Removal: place 50 μl ~ 500 μl fresh anti-coagulated blood sample to a clean 1.5 ml centrifuge tube,

centrifuge at 1000 g for 1 minute (for frozen blood, thaw the sample completely, and then operate as described above. The number of freeze-thaw cycles of blood sample should be less than three. If more than three, the samples will not get high quality DNA). And then carefully remove the supernatant containing about 1/2 of the total volume of blood.

a2. Red Blood Cells Removal: Add red blood cell lysis buffer (RCL Buffer) to the upper step solution, the volume of the RCL Buffer should be the same as the initial blood sample (if initial volume of whole blood used is 500 μl, the volume of RCL Buffer should be 500 μl), gently pipette up and down for 10 times to mix thoroughly, Incubate at room temperature for 5 minutes; and then proceed to step 2.

1B. For whole blood with nucleated erythrocytes (such as blood from fish, amphibian, reptile or bird): place 5 ~ 20 μl blood to a clean 1.5 ml centrifuge tube. Add 200 μl PBS, mix well and proceed to steps 2.

2. Centrifugation: Centrifuge at 1000 g for 1 minute. Remove the supernatant by aspiration carefully (be avoid of losing the precipitation).

3. Cell Lysis: Add 500 μl Lysis Buffer to the cell pellet, pipette up and down for 10 times to lyse the cells.

4. Vortex: Vortex for 10 seconds at high speed to completely lyse the cells.

DNA Binding

5. Transfer the cell lysates to the Spin Column. Centrifuge at 4000 g for 1 minute. Pour out the liquid in the collection tube, and then place the collection tube on absorbent paper to suck the liquid from the orifice of the tube. Put the Spin Column back into the collection tube.

Column Washing

6. Add 500 μl of Wash Buffer to the Spin column. Centrifuge at 12000 g for 1 minute. Discard the flow-through completely by aspirating.

7. Add 500 μl of 80% ethanol to the column. Centrifuge at 12000 g for 1 minute. And then add 300 μl of 80% ethanol to the column. Centrifuge at 12000 g for 1 minute(Important: be careful to avoid the bottom of the column touching with the flow-through when taking the column out from the collection tube, Otherwise the wash buffer will contaminate the DNA. If the liquid accidentally touched the bottom of the Spin Column, discard the liquid and place the column back to the collection tube, centrifuge for 1 minute to thoroughly remove the liquid on the column).

8. Transfer the column to a new 1.5 ml centrifuge tube, Open the lid and keep in the air for 2 minutes.

DNA Elution

9. Add 50 μl of Elution Buffer (20 ~ 100 μl, pre-heated to 60°C) to the center of the column, and incubate at room temperature for 2 minutes.

10. Centrifuge at 12000 g for 1 minute. Discard the column, determine the DNA concentration, do the following experiment with the purified DNA, or store the DNA at -20°C until needed. (For long-term preservation, it is recommended to place it at -80°C).

Representative Results

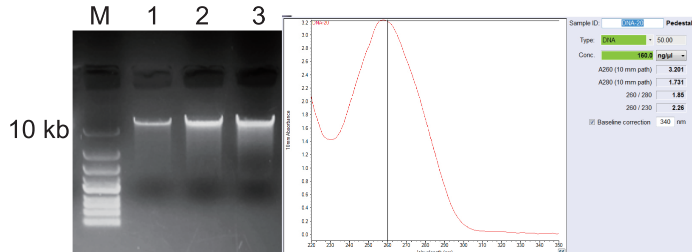


Figure 1. DNA isolated from 50 μl, 100 μl and 200 μl pig blood using *EZ*Bioscience® Blood DNA Purification Kit. DNA was eluted by 50 μl Elution Buffer, and 5 μl was loaded each lane. M: 250 bp DNA Ladder; Lane 1: DNA isolated from 50 μl blood; Lane 2: DNA isolated from 100 μl blood; Lane 3: DNA isolated from 200 μl blood. The UV absorbance of DNA purified from 200 μl pig blood (The DNA was eluted by 50 μl Elution Buffer).

Trouble Shooting

1. The DNA purity is not good enough.

a. Check whether ethanol is properly added to the reagent used, whether it is contaminated. Do the experiment with new products, and compare the results of the two groups. It is suggested that after the kit is opened, divide each buffer into 3 aliquots (which can be packed in 15 ml centrifugal tube). It should be strictly operated in accordance with the manual to prevent cross-contamination.

b. After centrifugation, the liquid in the collection tubes should be poured out and absorbent paper should be used to absorb the remaining liquid at the collection tube orifice. Operate strictly in the same way after each washing step.

C. Wash the column with Wash Buffer first, and then with 80% ethanol for twice. Be careful to avoid the lower side of the column touching with the flow-through when taking the column out from the collection tube after each wash step. Otherwise the wash buffer will contaminate the DNA. If the liquid accidentally touched the bottom of the Spin Column, discard the liquid and place the column back to the collection tube, centrifuge for 1 minute to thoroughly remove the liquid on the column.

2. The Yield of DNA is lower than expected.

a. Although 3 ~ 6 μg DNA can be purified from 100 μl mammalian blood, this doesn't mean that 30 ~ 60 μg DNA can be purified from 1 ml blood, because the binding efficiency of the column will decrease when too much blood is used. And more cells may be lost during the experiment.

b. Carry out the experiment as soon as the sample is ready. Do not leave the sample untreated too long before starting the experiment.

c. Sometimes, blood sample can be frozen and stored for a period. When using frozen blood, the number of freeze-thaw cycles must be less than 3, and the blood should not be stored for a too long time. The frozen blood sample can be incubated at 37°C and shaked from time to time until the sample is thawed completely.