- Low A260/A280 ratio for purified nucleic acids
  a) Inefficient cell lyses due to insufficient mixing of the sample with LB Buffer.
  b) Inefficient cell lysis due to the decreased protease activity.
  c) Not strictly operate according to the protocol. For example, inexact alcohol volume was added to Wash Buffer.
  d) Low- concentration alcohol was used instead of 100% pure alcohol.
  e) RNA contamination.

【Analysis DNA】

- Absorbance analysis

Take some DNA，dilute it into an appropriate concentration with elution Buffer.

Measure it at OD_{260} ， OD_{280} and OD_{320}.

Equation：concentration（μg/mL）＝50×OD_{260}×dilution multiple

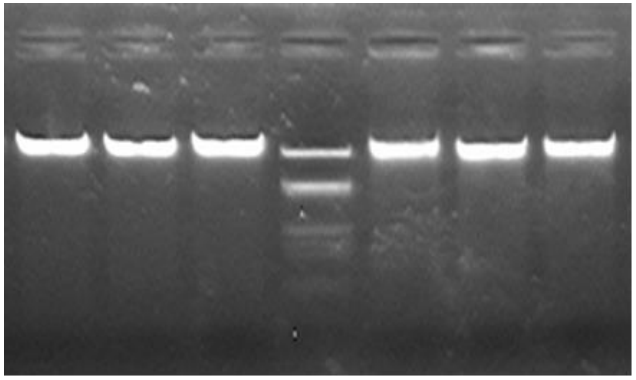
Target：2.0≥$OD_{260-320}$/ $OD_{280-320}$≥1.7

Notice: 1.0≥OD260≥0.1, the result of ratio is reliable.

- Agarose Gel Analysis

0.8～1％ Agarose gel

Example 1：Blood



【Company Information】

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Biospin Whole Blood Genomic DNA Extraction Kit

【Kit Components】

Cat#	BSC06S1	BSC06M1
Component	**50Tests**	**100Tests**
PK Solution	0.5mL	1.0 mL
LysisB Buffer	10.0mL	20.0 mL
WB1 Buffer	11.825mL (add 15.675mL ethanol before use)	23.65mL (add 31.35mL ethanol before use)
Wash Buffer	11.55mL×2 (add 17.325mL ethanol before use)	23.1mL×2 (add 34.65mL ethanol before use)
Elution Buffer	10mL	20mL
Spin column	50	100
Handbook	1copy	1copy

【Storage】

1. The PK Solution must be stored at 2～8℃, other components in the kit may be deposited at room temperature.
2. All components, when stored properly, can keep stable for 18 months.

【Introduction】

The kit provide a very simple, fast and effective technique for the isolation of pure high–molecular-weight genomic DNA in whole blood which was treated with anticoagulants such as citrate, heparin, EDTA. The kit is also suitable for the extraction of genome DNA from leukocytes. Based on the remarkable selectivity on genome DNA of Biospin membrane, the simple purification procedure involves only a few steps and allows isolation of high yields of pure genomic DNA within 30 minutes. No any expensive equipments are required, using of toxic or hazardous reagents, such as phenol or chloroform is completely avoided. In general, 2～6μg genomic DNA can be acquired from 200μL blood using the kit. The pure DNA can be applied extensively in PCR/Real-time PCR,sequencing,Southern blot,mutant analysis,SNP,and so on.

【Apparatus and Materials should Be Supplied by the User】

* 1.5mL sterile Micro-centrifuge tubes * 10μL/100μL/1000μL tips
* Micro-centrifuge capable of 14,000×g * Absolute ethanol (>99%)
* Vortex mixer

【Principle】

DNA in the sample is released using PK Solution and LysisB Buffer. The released DNA is bound exclusively and specifically to the Biospin membrane in presence of Lysis B Buffer and ethanol under appropriate salt iron and pH conditions. Denatured protein and other contaminants are removed with twice washing procedures. The DNA is then eluted from the membrane with the elution Buffer.

【Protocol】

According to the label on the bottle, add ethanol to WB1 Buffer and Wash Buffer.

The PK Solution should be stored at 2-8℃，take it out when using

1 Sample preparation：
   a) If the sample volume is more than 200μL，collect leukocytes by using erythrocyte lysis solution or Lymphocytes Separation solution，then resuspend using PBS Buffer , Mix well.
   b) If the sample volume is less than 200μL，adjust to 200μL by adding PBS Buffer and mix well.
   c) Other mammal blood DNA isolation method is same as human blood.
   d) For bird blood，the sample volume should be controlled within 20μL, and adjusted to 200μL by adding PBS Buffer and mix well.
2 Pipet 10μL of PK Solution into the bottom of a 1.5 or 2.0mL microcentrifuge tube.
3 Add 200μL of sample to the microcentrifuge tube from the step 2.
4 Add 200μL of the Lysis B Buffer to the sample from the step 3 and mix thoroughly for 5～10 seconds.
5 Incubate at 56℃ for 10 minutes.
6 Add 200μL of ethanol and mix thoroughly for 5～10 seconds.
7 Transfer all the mixture (in step 6) to spin column.
8 Centrifuge the mixture at 6,000~8,000×g for 1 minute. Discard flow-through.
9 Add 500μL of the WB1 Buffer to the Spin column. Centrifuge the Spin column at 10,000×g for 30～60 seconds. Discard flow-through.
10 Add 700μL Wash Buffer to the Spin column. Centrifuge at 10,000 ×g for 30～60 seconds. Discard flow-through.
11 Take the Spin column back to the tube.
12 Centrifuge the Spin column at 13,000 ×g for 2 minutes.
13 Place the Spin column in a new 1.5 or 2.0mL micro centrifuge tube (not provided).
14 Add 100μL the Elution Buffer. Incubate at room temperature for 1 minute. Centrifuge the mixture at 13,000×g for 1 minute. The DNA in the collection tube is ready for further analysis. If the isolated DNA sample is not going to be tested on the same day, freeze it at -20℃.

【Troubleshooting】

■ The amount of blood

The maximal volume of the samples should be within 1000μL, it is necessary to pool leukocytes with erythrocyte Lysis solution or Lymphocytes Separation solution when the sample volume is over 200μL.

■ Sample type

The kit is mostly used for human whole blood, or for other mammalian whole blood, and bird whole blood.

■ How to store the blood

The fresh whole blood should be stored at 2～8℃ or －20℃。If the blood was stored at －20℃, isolate the DNA after thawing.

■ Little or no DNA in the elution
   a) Low amount of leukocytes in the blood sample (For human or other mammal whole blood.)
   b) PK Solution did not be stored at 2～8℃, leading to its low activity .
   c) Forget adding ethanol to WB1 Buffer and Wash Buffer according to the label on the bottle before the first using.

■ For bird blood，after mixing the sample, PK Solution and Lysis B Buffer, the mixture is too ropy. Used too much bird blood, you should decrease the amount of the sample. Usually, bird blood is controlled within 20μL and adjusted to 200μL using PBS Buffer