Cat. # 9781

For Research Use

TaKaRa

# TaKaRa MiniBEST Whole Blood Genomic DNA Extraction Kit

Product Manual

v201310Da

# Table of Contents

## I. Description

TaKaRa MiniBEST Whole Blood Genomic DNA Extraction Kit is designed to purify genomic DNA from a variety of whole blood including 1 μl - 1 ml of whole blood with non-nucleated erythrocyte (such as mammals) and no more than 10 μl of whole blood with nucleated erythrocyte (such as bird and fish). The kit employs a unique lysis buffer for non-nucleated erythrocyte and leukocytes to release genomic DNA. And genomic DNA is efficiently purified from the biological sample in combination with DNA preparation membrane. The protocol provides a simple method to achieve the rapid isolation of highly purified genomic DNA and the entire procedure can be accomplished within 1 hour. Genomic DNA obtained is suitable for a variety of applications, such as PCR, Southern blotting, RAPD, AFLP, RFLP and other molecular experiments.

## II. Components (50 reactions)

The kit contains part I and part II.

Part I (stored at -20℃ )

| | |
|---|---|
| Proteinase K (20 mg/ml) | 1 ml |
| RNase A (10 mg/ml) | 500 μl |

Part II (stored at room Temperature (15 - 25℃ ))

| | |
|---|---|
| 10X Buffer RCL A＊1 | 2 ml x 2 |
| 10X Buffer RCL B＊1 | 16 ml |
| Buffer GB＊2 | 12 ml |
| Buffer WA＊2 | 28 ml |
| Buffer WB＊3 | 24 ml |
| Elution Buffer | 14 ml |
| Spin Column | 50 |
| Collection tubes | 50 |

＊ 1: The 10X Buffer RCL A and 10X Buffer RCL B are mixed in the ratio of 1:4 before use to prepare 10X Buffer RCL (erythrocyte lysis solution). The 10X Buffer RCL may be prepared by adding all 10X Buffer RCL A into 10X Buffer RCL B bottle before first use, and be stored at 4℃ for 1 year.

＊ 2: Contains Strong denaturant. Be careful to avoid contacting with skin and eyes. In the case of such contact, wash immediately with plenty water and seek medical advice.

＊ 3: Before using the kit, add 56 ml of 100% ethanol. Mix well.

**Reagents not supplied in this kit**

1. 100% ethanol
2. Sterile distilled water
3. PBS solution

## III. Storage and shipping

1. Part I can be stored and shipped at -20℃, Part II can be stored and shipped at room temperature (15 - 25℃).
2. 10X Buffer RCL A and 10X Buffer RCL B can be stored at room temperature. The mixed 10X Buffer RCL can be stored at 4℃ for 1 year.

## IV. Preparation before use

1. Adjust a water bath to 56℃.
2. Mix the 10X Buffer RCL A and 10X Buffer RCL B by the ratio of 1:4 to prepare the 10X Buffer RCL. 10X Buffer RCL was diluted 10 times with sterile distilled water before use. Prepare 1X Buffer RCL twice the volume of whole blood sample. The 1X Buffer RCL should be freshly prepared before use, do not stored for a long time.
3. Add 56 ml of 100% ethanol to Buffer WB and mix well before using it.
4. Pre-heat the Elution Buffer or sterile distilled water to 65℃ will improve elution efficiency.

## V. Protocol

For fresh whole blood, use 1X Buffer RCL.
Collect the leukocyte by centrifugation.
For frozen whole blood, collect the leukocyte by centrifugation.
When the volume of whole blood is less than 200 μl, add PBS up to 200 μl.

Add Buffer GB, Proteinase K, and RNase A, then incubate in 56℃ water bath.
Add 200 μl 100% ethanol and mix well.
Transfer the solution to Spin Column.

Discard the flow-through.

Buffer RWA
Buffer RWB

Discard the flow-through.

Place the Spin Column to 1.5 ml Tube.

Add Elution Buffer.

Genomic DNA

Figure 1. Flow chart

The procedure can be accomplished in 60 minutes. The whole procedure includes erythrocyte lysis, DNA binding with column, and DNA purification. Protocol in detail is as below.

**Sample preparation**

1. For whole blood with non-nucleated erythrocyte:
   - 200 μl - 1 ml fresh whole blood process to the steps 1 - 15 below.
   - 200 μl - 1 ml frozen whole blood is centrifuged at 2,000 rpm for 5 minutes, then 200 μl of the supernatant and precipitate will be used, and discard excess supernatant carefully. Process sample to Steps 7 - 15.

   In whole blood less than 200 μl, scale up to 200 μl with PBS and process to steps 7 - 15.
2. For whole blood with nucleated erythrocyte:
   Do not use more than 10 μl of whole blood. Add PBS up to 200 μl and proceed to steps 7 - 15.

**DNA Extraction**

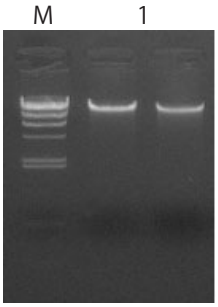
1. Prepare 1X Buffer RCL. First, mix 10X Buffer RCL A and 10X Buffer RCL B by the ratio of 1:4 to obtain 10X Buffer RCL. Then add sterilized water 9 times the volume of the 10X Buffer RCL to prepare 1X Buffer RCL. Prepare 1X Buffer RCL twice the volume of whole blood.
2. Add 1.5 volume of 1X Buffer RCL in the whole blood sample and mix well. Allowed to stand for 15 minutes at room temperature (15 - 25℃).
3. Centrifuge at 2,000 rpm for 5 minutes (Do not use greater centrifugal force). Discard the supernatant by decantation.
4. Add 1X Buffer RCL of 0.5 volume of the whole blood sample to the precipitate and mix well. The suspension was transferred to a 1.5 ml tube and stand at room temperature for 10 minutes.
5. Centrifuge at 2,000 rpm for 2 minutes (Do not use greater centrifugal force). Discard the supernatant carefully.
   Check whether the precipitate is obviously red color from erythrocyte or not. When it show red color, repeat Step 4 until there is no red color in the precipitate. (Most erythrocyte of mammalian whole blood by 2 times treatment can be clear fully, but blood with red cell having thicker membrane such as horse blood can be required more treatment).
6. Add 200 μl PBS in the precipitate to resuspend cells.
7. Add 200 μl of Buffer GB, 20 μl of Proteinase K (20 mg/ml), and 10 μl of RNase A (10 mg/ml). Mix well and incubate it in 56℃ water bath for 10 minutes.
8. Add 200 μl 100% ethanol and mix well.
9. Transfer the mixture to Spin Column. Centrifuge at 12,000 rpm for 2 minutes. Discard the flow-through.
10. Add 500 μl of Buffer WA into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
11. Add 700 μl of Buffer WB into Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
    Note: Make sure the amount of 100% ethanol specified on the bottle label has been added to the Buffer WB. Add Buffer WB along the tube wall of Spin Column to wash off any residual salt.
12. Repeat Step 11.

13. Place Spin Column in Collection Tube. Centrifuge at 12,000 rpm for 2 minutes.
14. Place Spin Column in a new 1.5 ml tube. Add 30 - 200 μl of Elution Buffer or sterile distilled water to the center of the membrane. Let it stand for 5 minutes at room temperature.
    Note: Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.
15. Centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
    If more yield is rquired, the elution can be re-applied into the center of the membrane, or add additional 30 - 200 μl of Elution Buffer or sterile distilled water and stand it for 5 minutes at room temperature and centrifuge at 12,000 rpm for 2 minutes to elute the DNA.
16. Quantification of the genomic DNA.
    The extracted genomic DNA can be quantitatively determined by electrophoresis or absorbance measuring.

## VI. Experimental examples

1. Purification of Genomic DNA from fresh horse whole blood

   Approximate 5 μg of highly purified genomic DNA was extracted from 1ml of fresh horse whole blood(containing $Na_2EDTA$ anticoagulant). The electrophoresis is in Figure 2.

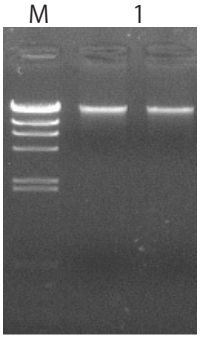



1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1 : Horse whole blood

Figure 2. Electrophoresis of Genomic DNA from horse whole blood

2. Purification of Genomic DNA from fresh pig whole blood

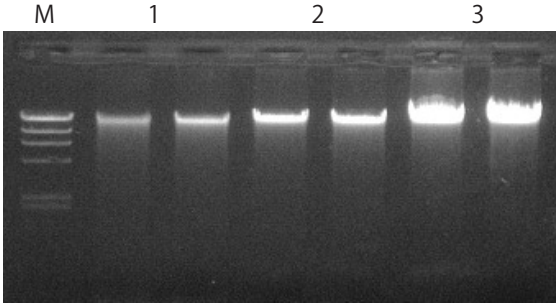
   Approximate 10 μg of highly purified genomic DNA was extracted from 1 ml of fresh pig whole blood (containing $Na_2EDTA$ anticoagulant). The electrophoresis is in Figure 3.




1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1 : Pig whole blood

Figure 3. Electrophoresis of Genomic DNA from pig whole blood

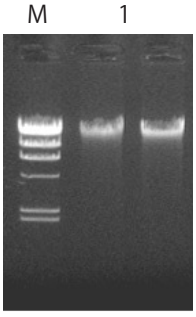
3. Purification of Genomic DNA from Whole Blood stored at -80℃. Different yield of genomic DNA were extracted from 1 ml of pig whole blood (containing $Na_2EDTA$ anticoagulant) treated with different cycle of freeze-thaw. The electrophoresis is in Figure 4.




1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1 : Pig whole blood (freeze-thaw twice) (2 μg DNA was obtained)
2 : Pig whole blood (freeze-thaw once) (8 μg DNA was obtained)
3 : Pig whole blood (fresh) (10 μg DNA was obtained)

Figure 4. Electrophoresis of Genomic DNA from frozen pig whole blood

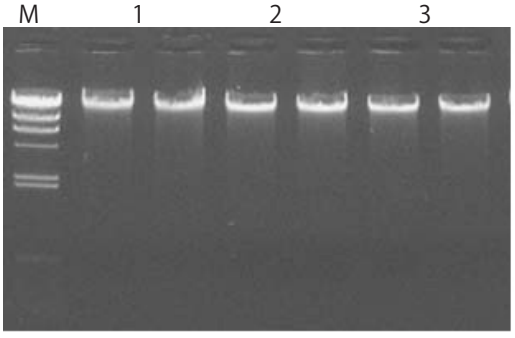
4. Purification of Genomic DNA from fresh fish whole blood
Approximate 12 μg of highly purified genomic DNA was extracted from 5 μl of fresh fish whole blood (containing Na2EDTA anticoagulant). The electrophoresis is in Figure 5.




1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1: Fish whole blood

Figure 5. Electrophoresis of Genomic DNA from fresh fish whole blood

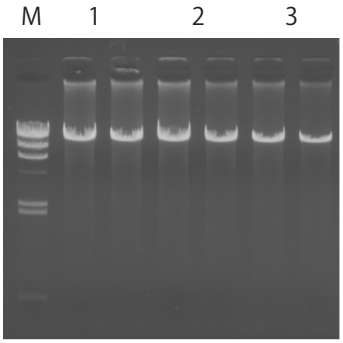
5. Purification of Genomic DNA from fresh human whole blood
Approximate 5 μg of highly purified genomic DNA was extracted from 1 ml of human whole blood (containing sodium citrate, $Na_2EDTA$, or heparin anticoagulant). The electrophoresis is in Figure 6.




1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1 : Human whole blood containing Sodium citrate anticoagulant
2 : Human whole blood containing $Na_2EDTA$ anticoagulant
3 : Human whole blood containing heparin anticoagulant

Figure 6. Electrophoresis of Genomic DNA from fresh human whole blood containing different anticoagulant

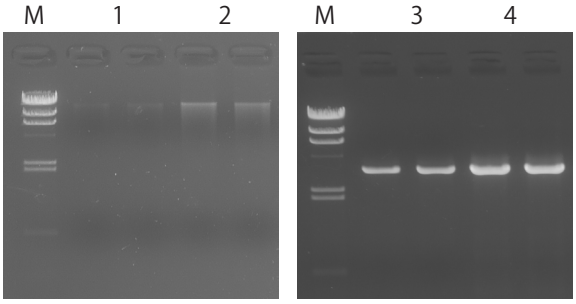
6. Purification of Genomic DNA from human whole blood and PCR amplification. Approximate 5 μg of highly purified genomic DNA was extracted from 1ml of human whole blood. Using the genomic DNA obtained as template, 17.5 kb of β -globin gene fragment was amplified by PCR. The electrophoresis is in Figure 7.




1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1 : PCR product of human whole blood containing heparin anticoagulant genomic DNA
2 : PCR product of human whole blood containing $Na_2EDTA$ anticoagulant genomic DNA
3 : PCR product of human whole blood containing Sodium citrate anticoagulant genomic DNA

Figure 7. Electrophoresis of PCR fragment amplified using genomic DNA obtained

7. Purification of Genomic DNA from 1 μl and 5 μl of mouse whole blood and PCR amplification of 3 kb fragment of *B2m* gene.




1% Agarose gel electrophoresis
M : λ -*Hin*d III digest
1 : Genomic DNA from 1 μl mouse whole blood
2 : Genomic DNA from 5 μl mouse whole blood
3 : PCR product of genomic DNA from 1 μl mouse whole blood
4 : PCR product of genomic DNA from 5 μl mouse whole blood

Figure 8. Electrophoresis of Genomic DNA from mouse whole blood and 3 kb PCR fragment

## VII. Cautions

1. Use fresh whole blood and avoid repeated freeze and thawing to ensure the extracted genomic DNA not to be degraded.
2. Preservation of the blood sample.
   (1) Short-term preservation: Blood samples with anticoagulant can be stored at 2 - 8℃ for up to 10 days. For some experiments such as Southern hybridization, which required the full length genomic DNA, blood samples should be stored for no more than 3 days.
   (2) Long-term preservation: Blood samples with anticoagulant should be stored in the -70℃. Avoid repeated freeze and thawing.
3. Some reagents contain chemical irritants. When working with these reagents, always wear suitable protection such as safety glasses, laboratory coat and gloves and try to operate at fume hood. Be careful to avoid contacting with eyes and skin. In the case of such contact, wash immediately with plenty water and seek medical advice.
4. When genomic DNA is stored for a long time, it is recommended to save it in the Elution Buffer.
5. Standard operation applies to human whole blood materials. Other mammalian whole blood is also basically the same operation. However, the procedure of the lysis of erythrocyte is not the same. For examples, the horse blood and other materials need to be processed multiple times for complete erythrocyte lysis because erythrocyte membrane is thicker, and small animals may need to reduce degradation time and frequency.
6. Be sure not to use too much centrifugal force in centrifugation, because the leukocyte will be damaged and suspended normally.
7. Mix the 10X Buffer RCL A and 10X Buffer RCL B by the ratio of 1:4 to obtain the 10X Buffer RCL. Freshly prepare before use or mix 10X Buffer RCL A and 10X Buffer RCL B all together at first use and then stored at 4℃ for one year.
8. Be sure whether the red cells are non-nucleated in whole blood. If the red cell is nucleated, the starting amount over 10 μl may cause clog in Spin Column.

## VIII. Troubleshooting

Q1. How extraction yield of genomic DNA?

A1. The kit is suitable for purification of genomic DNA from fresh or frozen whole blood no more than 1 ml volume. The amount of DNA extracted from fresh whole blood material is more. Generally, 5 μg of genomic DNA can be extracted from 1 ml of horse whole blood; 10 μg of DNA can be from 1 ml of pig whole blood; 5 μg of DNA can be from 1 ml of human whole blood; 12 μg of DNA can be from 5 μl of fish whole blood. When frozen, repeated freeze-thawed, or long-period stored whole blood material are extracted, the recovery of DNA will be significantly reduced.

Q2. When genomic DNA is no or low yield.

A2. (1) Pre-heat the Elution Buffer or sterile distilled water at 65℃ can improve elution efficiency.

(2) The starting amount is lower or whole blood is not fresh enough (to avoid repeated freeze-thawing).

(3) Strictly follow the protocol.

Q3. When the extracted genomic DNA is degraded.

A3. (1) The whole blood is not fresh enough. Blood samples with anticoagulant can be stored for 10 days at 2 - 8 ℃

(2) After adding Buffer RCL, standing too long time would cause extracted genomic DNA degradation.

(3) Erythrocyte lysis is not sufficient. When red cells remain in the precipitate after adding Buffer RCL, degradation of the precipitate using 1X Buffer RCL should be repeated.

Q4. When there is RNA contamination in extracted genomic DNA.

A4. (1) Follow the protocol to use RNase A.

(2) RNase A may be inactivated. RNase A should be stored at -20℃.

Q5. When the extracted genomic DNA have low biological activity.

A5. (1) Salt concentration in extracted genomic DNA is too high. When washing the DNA preparation membrane with Buffer WA and Buffer WB, add these along the tube wall of Spin Column and stand for 5 minutes at room temperature to wash off any residual salt.

(2) There is residual ethanol in genomic DNA solution. Stand the Spin Column for 2 minutes at room temperature before adding Elution Buffer to the Spin Column and it will improve the effect of elution.

(3) The Elution Buffer must be added in the center of membrane during DNA eluting and not residue on the tube wall of Spin Column.

Q6. What is the difference in amount of DNA extraction between frozen whole blood and fresh whole blood?

A6. Whole blood after frozen will make the most of the erythrocytes and leukocyte broken. Since DNA is mainly present in the leukocytes, Usually the amount of DNA extracted from frozen whole blood will decrease by 20% to 50%.

**NOTE :** This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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