Cat. # 9782

For Research Use

TaKaRa

TaKaRa MiniBEST FFPE DNA Extraction Kit

Product Manual

v201610Da

Table of Contents

I. Description

The TaKaRa MiniBEST FFPE DNA Extraction Kit is designed to purify genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples. This kit uses special paraffin removal and lysis buffers, in combination with a spin column containing a DNA preparation membrane to efficiently purify genomic DNA from FFPE samples. The protocol provides a simple method for rapid isolation of highly purified genomic DNA within 2 hr. Genomic DNA prepared by this method is suitable for a variety of applications, such as PCR, real-time PCR, SNP analysis, and other molecular biology applications.

II. Components

The kit contains Part I and Part II.

Part I (stored at -20℃)

1.	Proteinase K (20 mg/ml)	1 ml
2.	RNase A (10 mg/ml)	0.5 ml

Part II (stored at room temperature)

1.	Buffer DP	28 ml
2.	Buffer GL*1	12 ml
3.	Buffer GB*1	12 ml
4.	Buffer WA*1	28 ml
5.	Buffer WB*2	24 ml
6.	Elution Buffer	14 ml
7.	Spin Columns	50
8.	Collection tubes	50

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

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

Reagents not supplied in this kit

1. 100% ethanol
2. Sterile purified water
3. PBS Solution

III. Storage and shipping

1. Part I can be stored at -20℃, and Part II can be stored at room temperature (15 - 25℃).
2. Part I can be shipped at -20℃, and Part II can be shipped at room temperature (15 - 25℃).

IV. Preparation before use

1. Set a water bath to 56℃, and a second water bath or a heating block to 80℃.
2. Add 56 ml of 100% ethanol to Buffer WB and mix well.
3. Preheat the Elution Buffer or sterile purified water to 65℃ to improve elution efficiency.

V. Protocol

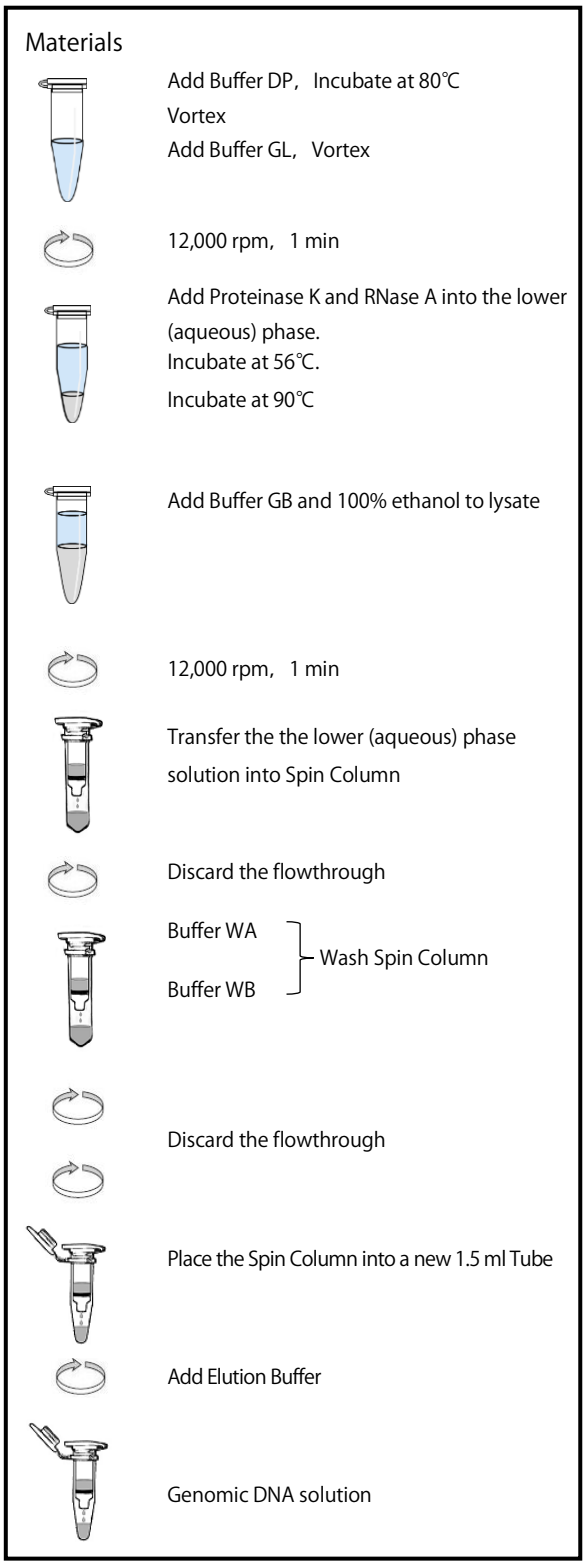


Figure 1. Protocol overview

An overview of the protocol is shown in Figure 1. It can be completed in about 2 hr, and includes deparaffinization, lysis, DNA binding to a Spin Column, and DNA purification. The protocol is described in detail below.

1. Sample preparation

   Using a sterilized scalpel, cut up to 7 sections that are 5-10 μm thick and about 250 mm^2 in area, and place the sections in a 1.5 or 2 ml microcentrifuge tube (not supplied). If the sample surface has been exposed to air, discard the first 2–3 sections.

   If extracting DNA from tissue sections that have been applied to microscope slides, use a sterile blade to scrape the sections from the slide.
2. Add 500 μl of Buffer DP to the sample, close the lid, and vortex vigorously for 10 sec.
3. Incubate 1 min at 80℃, then vortex the sample immediately at a vigorous speed to mix.

   Cool down the sample to room temperature.
4. Add 180 μl of Buffer GL to the sample, and vortex vigorously for 10 sec.
5. Spin at 12,000 rpm for 1 min at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (organic) phase.
6. Pipette 20 μl Proteinase K and 10 μl RNase A directly into the lower (aqueous) phase.

   Mix the aqueous phase by pipetting up and down several times. Pipette only the lower (aqueous) phase up and down. Avoid mixing the lower and upper phases excessively.
7. Incubate 1 hr at 56℃.

   Note: Set a water bath or heat block to 90℃ for next step.
8. Incubate 30 min at 90℃, then cool down the sample to room temperature.
9. Add 200 μl of Buffer GB and 200 μl 100% ethanol to the sample and mix well.
10. Spin at 12,000 rpm for 1 min at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (organic) phase.
11. Place a Spin Column into a Collection Tube. Transfer the lower (aqueous) phase solution to the Spin Column. Centrifuge at 12,000 rpm for 2 min. Discard the flowthrough.
12. Add 500 μl of Buffer WA to the Spin Column. Centrifuge at 12,000 rpm for 1 min. Discard the flowthrough.
13. Add 500 μl of Buffer WB to the Spin Column. Centrifuge at 12,000 rpm for 1 min. Discard the flowthrough.

    Note: Make sure the amount of 100% ethanol specified on the bottle label has been added to Buffer WB. Then add Buffer WB along the tube wall of the Spin Column to wash off any residual salt.
14. Repeat Step 13.
15. Place the Spin Column into a Collection Tube. Centrifuge at 12,000 rpm for 2 min.

16. Place the Spin Column into a new 1.5 ml tube (not supplied) . Add 50-100 μl of Elution Buffer or purified water to the center of the membrane. Let it stand for 5 min at room temperature.

    Note: Preheating the Elution Buffer or purified water at 65℃ can improve elution efficiency.

17. Centrifuge at 12,000 rpm for 2 min to elute the DNA.

    Note: If a higher yield is needed, reload the eluted DNA solution onto the center of the Spin Column membrane, or add 50-100 μl of Elution Buffer or purified water, let it stand for 5 min at room temperature, and centrifuge at 12,000 rpm for 2 min to elute the DNA.

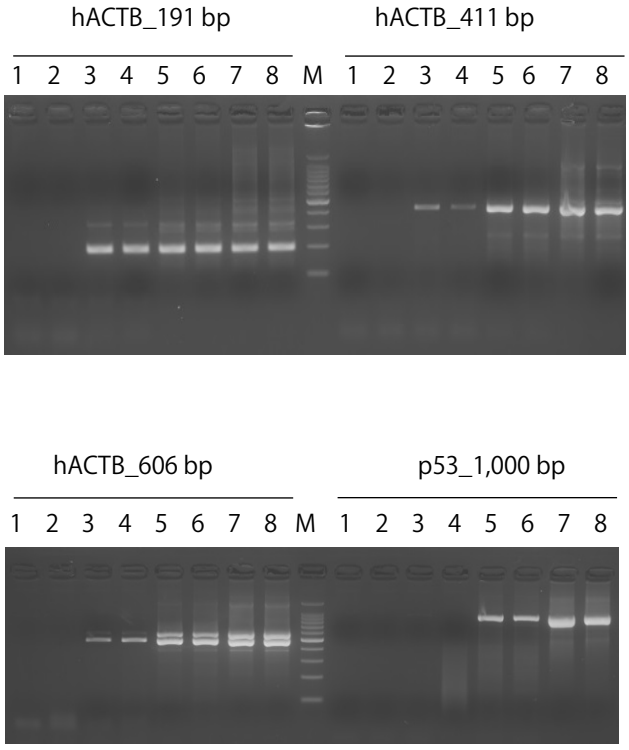
18. Quantification of the genomic DNA.

    The extracted genomic DNA can be quantified by electrophoresis or absorbance measurements.

VI. Experimental examples

Purification of genomic DNA from human tonsil FFPE samples, and PCR applications

Genomic DNA was purified from 2 sections of human tonsil FFPE samples (10 μm thick and 200 mm^2 in area). Using genomic DNA as template, 191 bp, 411 bp, and 606 bp gene fragments, and a 1 kb p53 gene fragment were amplified with MightyAmp™ DNA Polymerase Ver.3 (Cat. # R076A) and analyzed using agarose gel electrophoresis (Figure 2).



M：100 bp DNA Ladder（Dye Plus）
1, 2: Negative Control
3, 4: Direct PCR with FFPE samples
5, 6: PCR with purified genomic DNA
7,8：Positive Control

Figure 2. Electrophoresis of PCR fragments using genomic DNA from human tonsil FFPE samples.

VII. Precautions for use

1. DNA yield and quality are largely dependent on the type, quality, quantity, and length of time of storage of FFPE samples.
2. Remove as much excess paraffin as possible from the sample for a successful extraction.
3. Some reagents contain chemical irritants. When working with these reagents, always wear suitable protective gear such as safety glasses, a laboratory coat, and gloves, and work in a fume hood. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.
4. If genomic DNA is stored for a long time, it should stored in Elution Buffer.
5. Do not use more than the recommended maximum amount of FFPE sample, and make sure the sample is completely lysed, in order to avoid reducing the yield and blocking the Spin Columns.
6. If Spin Column clogging occurs, you can increase the centrifugal force to 15,000 rpm, and extend the centrifugation time.
7. If the tissue lysate is not clear and homogeneous, add double the volume of Buffer GL, Proteinase K, and RNase A to continue the lysis.

VIII. Troubleshooting

Q1. Why is little or no genomic DNA isolated?

A1. Possible explanations and solutions include:

(1) The amount of genomic DNA in the starting material is low, so increasing the amount of starting material will help.

(2) Incomplete lysis of the tissue causes incomplete release of DNA. We recommend extending the lysis time or increasing the amount of lysis buffer.

(3) Excessive starting material makes it difficult to achieve complete lysis. Increase the amount of lysis buffer appropriately or divide the starting material into multiple portions for extraction.

(4) Preheating the Elution Buffer or sterile purified water to 65℃ can improve elution efficiency.

(5) Strictly follow the protocol.

Q2. Why can't the extracted genomic DNA be used to amplify long PCR targets?

A2. The quality of DNA extracted by this kit strongly depends on sample type, quality, quantity, and the amount of time the sample has been stored. If the FFPE sample is too old (> 1 year), PCR targets larger than 1 kb may not be amplified.

Q3. Why is the extracted genomic DNA low in purity ?

A3. Possible explanations and solutions include:
(1) The salt concentration of the extracted genomic DNA is too high. When washing the column membrane using Buffer WA and Buffer WB, add these buffers along the tube wall of the Spin Column and let it stand for 5 min at room temperature to wash off any residual salt and increase the effectiveness of the wash.
(2) There is residual ethanol in the lysate. Incubate the column for 2 min at room temperature before adding Elution Buffer to the column.
(3) The Elution Buffer must be added to the center of the Spin Column membrane, not the residue on the tube wall of the column.

Q4. Why is the extracted genomic DNA contaminated with RNA?

A4. (1) RNase A was not used in the protocol. Strictly follow the protocol, making sure to use RNase A.
(2) The RNase A may be inactivated. RNase A should be stored at -20℃. RNase A is generally stable and not easily inactivated.

Q5. What should I do if the amount of starting material is more than the amount described in this protocol?

A5. This kit is designed to purify a small amount of genomic DNA. If the amount of starting material is more than the amount in the protocol, increase the amount of Buffer GL and Buffer GB and divide the homogenate into two Collection Tubes. It is better to limit the amount of starting material so it falls within the specified range and divide it into multiple portions for extraction, than to use excessive amounts of starting material, which will limit the extent of lysis and the yield of DNA. Otherwise, the resulting incomplete lysis will block the column and cause the experiment to fail.

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