Cat. # 9762

For Research Use

TaKaRa

TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0

Product Manual

v201811Da

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I. Description

TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 is designed for rapid purification of DNA fragment from agarose gel. The kit employs a proprietary buffer for solubilizing agarose gels in combination with a convenient column to purify DNA fragments. The buffer has strong buffering properties and contains a pH indicator which makes it easy to determine whether the pH value is suitable for combination with Spin column membrane. In addition, the buffer can solve a gel quickly at the room temperature (15 - 25℃) without heating. The entire procedure can be accomplished within 20 minutes. The Spin Column can bind up to 20 μg of DNA and efficiently recovered DNA fragments in a size range of 50 bp - 20 kb. The recovery rate is approximately 50 - 80%. However, the recovery rate will be lower if DNA fragments are in a size range of 20 - 50 kb. The DNA fragments purified by this kit are suitable for all routine molecular biology applications, such as ligation, PCR, sequencing, etc.

II. Components (50 reactions)

One reaction can handle 300 mg of agarose gel (gel concentration is 1%).
The kit contains Reagent Set and Column Set.

Reagent Set

Buffer GM*1	50 ml
Buffer WB*2	24 ml
Elution Buffer	2 ml x 2

＊1 contain 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.

＊2 Before using the kit, add 56 ml of 100% ethanol to Buffer WB.

Column Set

Spin Columns	50
Collection tubes	50

[Reagents not supplied in this kit]

1. 100% ethanol
2. Sterile purified water or Tris-HCl (pH 8.0)
3. 3 M sodium acetate (pH 5.2)

III. Shipping and Storage

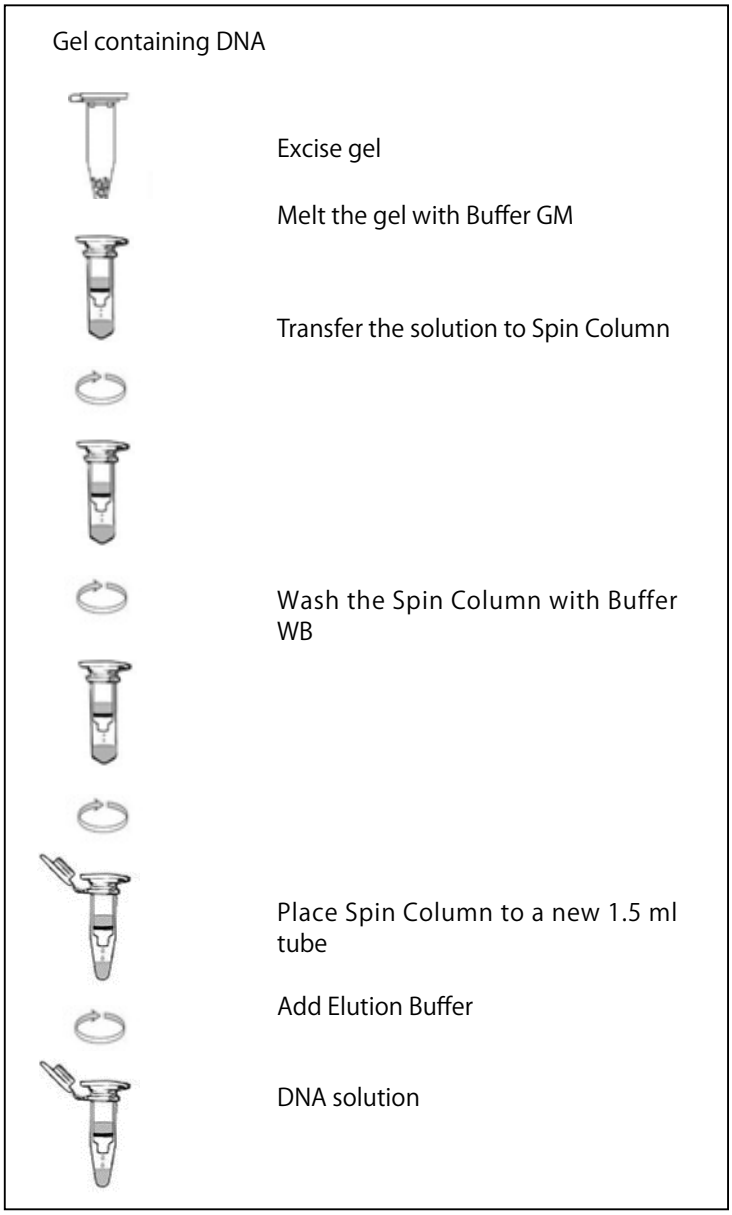
1. The kit can be stored at room temperature (15 - 25℃).
2. The kit is shipped at room temperature (15 - 25℃).
3. Precipitate may occur in the Buffer GM under low temperature. Please warm with a 37℃ to dissolve and use after returning to room temperature.

IV. Preparation before Use

1. Before the first use of the kit, add 56 ml of 100% ethanol to Buffer WB.
2. Confirm the Buffer GM is still yellow.
3. The reagent contains strong denaturant. Be careful to avoid contacting with skin and eyes.

V. Protocol

● Flow chart



The entire procedure can be accomplished within 20 minutes.
Protocol in detail is as below.

1. Make agarose gel with TAE or TBE buffer and apply target DNA in agarose gel electrophoresis.
2. Excise the agarose gel slice containing the DNA fragment with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Cut as close to the DNA as possible to minimize the gel volume and increase the recovery rate of DNA. If the gel is more than 300 mg, use more than one Spin Column as the recovery rate will be seriously affected.
   Note: DNA should not be exposed to ultraviolet illumination for a long time in case of DNA damage.
3. Cut the gel into small pieces by cutting the gel. The gel melting time in Step 6 can be shortened and the recovery rate of DNA can be increased.
4. Weigh the gel pieces and calculate the volume of gel. 1 mg of gel is equivalent to a 1 μl volume.
5. Add Buffer GM to gel for melting. The amount of Buffer GM is shown in the table below.

gel concentration	Buffer GM
1.0%	3X sample volume
1.0 - 1.5%	4X sample volume
1.5 - 2.0%	5X sample volume

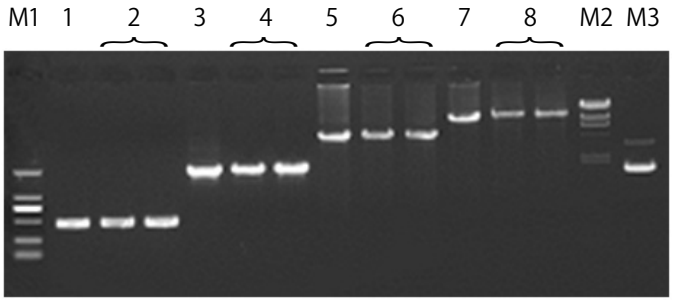
6. Mix well and melt the gel at room temperature (15 - 25℃). If the concentration of gel is too high or the gel is hard to melt, warm at 37℃. Intermittent vortexing will accelerate gel solubilization.
   Note: Gel must be completely dissolved, or the DNA fragment recovery will be reduced. Extend the melting time when the gel concentration is high.
7. After the gel has been completely molten, check the color of the solution. If the color becomes orange or pink from yellow, add 10 μl of 3 M sodium acetate (pH 5.2) to the solution and vortex well untill the solution return to yellow. When the DNA fragment is smaller than 400 bp, add further with isopropanol to final concentration of 20%.
8. Set a Spin Column into Collection Tube.
9. Transfer the solubilized agarose from Step 7 into the column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
   Note: For improvement the recovery rate of DNA, transfer the flow-through to Spin Column again and centrifuge again.
10. Add 700 μl of Buffer WB into the Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
    Note: Make sure that the amount of 100% ethanol specified on the bottle label has been added to the Buffer WB.
11. Repeat Step 10.
12. Place the Spin Column back into Collection Tube. Centrifuge at 12,000 rpm for 1 minute.
13. Place the Spin Column into a new 1.5 ml tube. Add 30 μl of Elution Buffer or Sterile purified water to the center of the membrane. Let it stand for 1 minute at room temperature.
    Note: Pre-heat the Elution Buffer or Sterile purified water to 60℃ can improve elution efficiency.
14. Centrifuge at 12,000 rpm for 1 minute to elute the DNA.

If there is negative pressure device suitable for Spin Column interface, operate following protocol after Step 7 above-mentioned.

8. Attach Spin Column to the interface negative pressure device.
9. Transfer the solubilized agarose from Step 7 to the Spin Column. Switch on the vacuum source and adjust the negative pressure (flow rate controlled at 1 drop/second). Continue to apply vacuum slowly until no liquid remains in the Spin Column.
10. Pipette 700 μl of Buffer WB into the Spin Column. Draw all liquid through the column.
    Note: Make sure that the amount of 100% ethanol specified on the bottle label has been added to the Buffer WB.
11. Repeat the Step 10. Transfer the Spin Column into a Collection Tube.
12. Centrifuge at 12,000 rpm for 1 minute.
13. Place the Spin Column into a new 1.5 ml tube. Add 30 μl of Elution Buffer or Sterile purified water to the center of the membrane. Let it stand for 1 minute at room temperature.
    Note: Pre-heat the Elution Buffer or Sterile purified water to 60℃ can improve elution efficiency.
14. Centrifuge at 12,000 rpm for 1 minute to elute the DNA.

VI. Experimental Examples

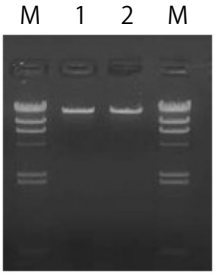
Example 1: Perform 1% agarose gel electrophoresis using 3 μg of 500 bp PCR fragment, 3 μg of 2 kb PCR fragment, 2 μg of 5 kb PCR fragment and 2 μg of 10 kb PCR fragment. After electrophoresis, purify the DNA fragments using this kit and then analyze 1/10 volume of the purified DNA by agarose gel electrophoresis (Figure 1). The recovery ratio is about 60%.



1% Agarose gel electrophoresis
M1： DL2,000 DNA Marker
1： 500 bp DNA unpurified
2： 500 bp DNA purified
3： 2 kb DNA unpurified
4： 2 kb DNA purified
5： 5 kb DNA unpurified
6： 5 kb DNA purified
7： 10 kb DNA unpurified
8： 10 kb DNA purified
M2： λ-*Hin*d III digest
M3： pUC119 DNA

Figure 1. Electrophoresis of purified DNA fragment

Example 2: Perform 1% agarose gel electrophoresis using 4 μg of 18 kb PCR fragment. After electrophoresis, purify the DNA fragments and then analyze 1/10 volume of the purified DNA by agarose gel electrophoresis (Figure 2). The recovery ratio is about 50%.



M: λ-*Hin*d lll digest
1: 18 kb DNA unpurified
2: 18 kb DNA purified

Figure 2. Electrophoresis of purified DNA fragment

VII. Cautions

1. Make sure that the amount of ethanol specified on the bottle label has been added to the Buffer WB.
2. Make sure the gel is not too large. Minimize the gel volume, because gel volume will affect the yield of DNA.
3. It is recommended to elute DNA with Elution Buffer if the purified DNA is for long-time storage.
4. It is recommended to elute DNA in Sterile purified water if the purified DNA is for sequencing.

VIII. Troubleshooting

Q1. How much Elution Buffer need to purify DNA?
A1. The amount of Elution Buffer can be calculated according to the required concentration. Generally, more than 90% DNA can be purified using more than 30 μl of Elution Buffer, so the amount of Elution Buffer should be more than 30 μl. When the required concentration of DNA is higher, the amount of Elution Buffer can be reduce to 20 μl with a lower purification efficiency which can be increased by pre-warming the Elution Buffer and letting the tube stand for 1 minute before centrifuge.

Q2. How amount of DNA is purified by this kit?
A2. Each column has a binding capacity of up to 20 μg of DNA fragments and the least amount of DNA is 500 ng. Please estimate the total amount of DNA sample before using the kit.

Q3. The yield of DNA is low, why?
A3. The recovery efficiency of DNA is 50 - 80%. When the yield of DNA is lower, consider the following aspects:
(1) Gel volume was too large (>300 mg). 50 - 80% recovery of DNA can only be obtained from 300 mg gel per column. If gel is >300 mg, you should use multiple columns.
(2) Make sure that the volume of 100% ethanol specified on the bottle label has been added to the Buffer WB.

(3) Gel slice is incompletely dissolved. Pay attention to whether the gel slice is dissolved completely or not during melting. The incompletely dissolved gel slice would affect the yield of DNA. Inverting the tube occasionally help to increase the melting of gel slice.
(4) The pH in the melting solution is higher. Change of the color of melting solution from yellow to orange or purple indicate that the pH in the melting solution is higher. Add 10 μl of 3 M sodium acetate (pH5.2), mix well until the color of the melting solution turn back to yellow, and then continue with the procedure.
(5) Centrifugation at room temperature can improve to binding DNA to the membrane of the Spin Column.
(6) The centrifugation before eluting should not be omitted. It can remove the residual ethanol in the Column. The elution efficiency will be affected by the residual ethanol.
(7) Heat the Elution Buffer or Sterile purified water to 60℃ can improve elution efficiency.

Q4. How to extract large DNA fragments from agarose gel?

A4. When the size of DNA fragments is long (more than 10 kb), the recovery of the DNA will be lower. The following aspects can be considered:
(1) Increasing the starting amount of up to 2 - 5 μg
(2) Pre-heat the Elution Buffer at 60℃ can improve elution efficiency
(3) Reduce the physical damage the operation of large DNA fragments, such as the vortex; DNA should not be exposed to ultraviolet illumination for a long time; use 6X Quadricolor-loading Buffer (Cat. #9171) for electrophoresis; avoid the use of repeated exposure of the ultraviolet illumination.

Q5. The purified DNA have low biological activity.

A5. (1) There is residual salt ion in the eluate. Let it stand at room temperature for 5 minutes after adding Buffer WB to remove the residual salt ion in the column completely.
(2) There is residual ethanol in eluate. Before adding the eluate in the Spin Column, let it stand at room temperature for 2 minutes to help the residual ethanol to evaporate.
(3) Use Sterile purified water for eluting DNA.