Cat. # 9761

For Research Use

TaKaRa

TaKaRa MiniBEST DNA Fragment Purification Kit Ver.4.0

Product Manual

v201306Da

Table of Contents

I. Description

TaKaRa MiniBEST DNA Fragment Purification Kit Ver.4.0 is designed for purify DNA fragments from PCR and other enzymatic reactions and the protocol will remove unincorporated primers (< 65 nt), enzymes and nucleotides. The kit employs a special binding solution in combination with a column having an optimized silica membrane to achieve high selectivity and recovery of DNA fragments. The whole procedure can be finished within 15 minutes. Each column has a binding capacity of up to 20 μg of DNA fragments (50 bp - 10 kb) with an expected recovery of 70 - 95%. The efficiency of recovery of DNA fragment larger than 20 kb (20 - 50 kb) is low. The purified DNA fragments are with high purity and without enzymes, DNA primers or dNTP and suitable for a variety of downstream applications.

II. Kit Components (50 reactions)

This kit contains reagent set and column set.

Reagent Set

Buffer DC*1	28 ml
Buffer WB*2	24 ml
Elution Buffer	2 ml x 2

＊1: Contain strong denaturant. Be careful to avoid contacting with eyes and skin. In the case of such contact, wash immediately with plenty water and seek medial advice.
＊2: Before the first use of 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】
100% Ethanol
Sterilized water or Tris-HCl (pH8.0)

III. Storage and Shipping

1. Store the kit at room temperature (15 - 25℃).
2. Ship the kit at room temperature (15 - 25℃).

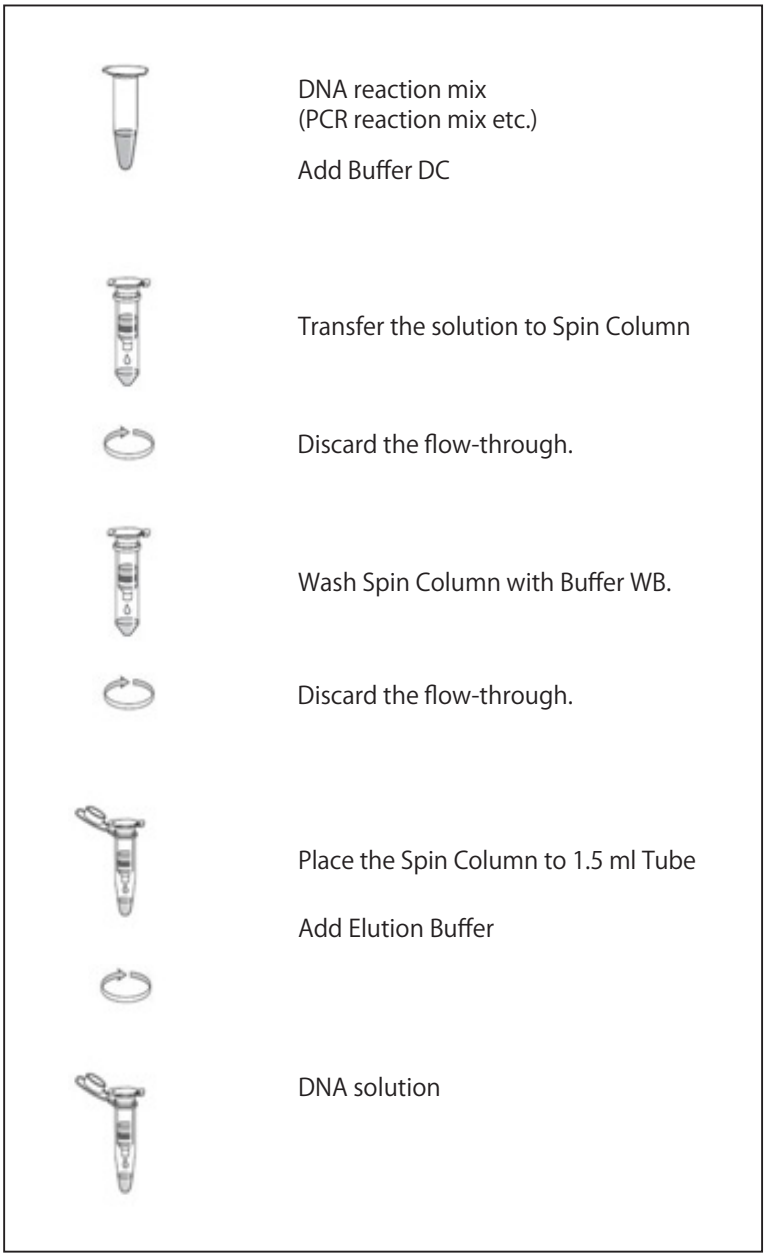
IV. Preparation before Use

1. Before the first use of the kit, add 56 ml of 100% ethanol to Buffer WB.
2. Reagents contain strong denaturant. When working with these reagents, always wear suitable protection such as laboratory coat and gloves.

V. Protocol

The protocol overview is below. The entire procedure can be finished in 15 minutes.

● Flow chart



Protocol in detail is as below.

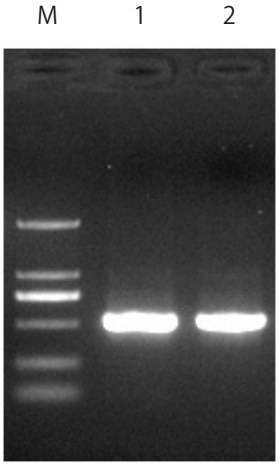
1. Add a 3X volume of Buffer DC to the PCR or other enzymatic reactions. If the required volume of Buffer DC is less than 100 μl, add 100 μl of Buffer DC. Vortex briefly to mix the sample.
2. Place a Spin Column into a Collection Tube.
3. Pipette the sample from Step 1 into the Spin Column. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
   Note: For improvement the recovery of DNA, transfer the flow-through to Spin Column and centrifuge again.
4. Add 700 μl Buffer WB to Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
   Note: Make sure that the volume of 100% ethanol indicated on the bottle label has been added to the Buffer WB.
5. Repeat Step 4.
6. Place the Spin Column back into the Collection Tube. Centrifuge at 12,000 rpm for 1 minute.
7. Place the Spin Column into a new clean 1.5 ml tube. Add 25 - 30 μl Elution Buffer or sterilized water to the center of the membrane. Let it stand for 1 minute at room temperature.
   Note: Pre-heat the Elution Buffer or sterilized water to 60℃ can increase the elution efficiency.
8. Centrifuge at 12,000 rpm for 1 minute at room temperature to elute DNA.

If there is negative pressure device suitable for Spin Column interface, operate the following protocol after Step 1 mentioned above.

2. Attach the vacuum manifold to the vacuum source. Insert a Spin Column into one of the complimentary fittings. Pipette the sample from Step1 to a Spin Column. Switch on vacuum source and adjust the negative pressure (flow rate controlled at 1 drop/second). Continue to apply vacuum until no liquid remains in the Spin Column.
3. Adjust the negative pressure to the maximum. Pipette 700 μl of Buffer WB into the Spin Column. Apply vacuum until no fluid remains in the Spin Column.
   Note: Make sure that the amount of 100% ethanol indicated on the bottle label has been added to the Buffer WB.
4. Repeat the Step 3. Remove the Spin Column from negative pressure device and place to the Collection Tube.
5. Centrifuge at 12,000 rpm for 1 minute.
6. Place the Spin Column into a new clean 1.5 ml tube. Add 25 - 30 μl of Elution Buffer or sterilized water to the center of the membrane. Let it stand for 1 minute at room temperature.
   Note: Pre-heat the Elution Buffer or sterilized water to 60℃ can increase the elution efficiency.
7. Centrifuge at 12,000 rpm for 1 minute at room temperature to elute the DNA.

VI. Experimental Example

1. Amplify 500 bp DNA fragment by PCR and purify the DNA fragment by this kit. The result of agarose gel electrophoresis shows in Fig. 1. The recovery of DNA fragment is up to 90%.

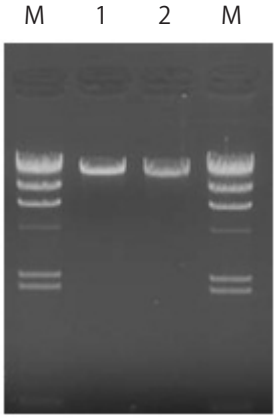


1% Agarose gel electrophoresis

M : DL2,000 DNA Marker
1 : 500 bp DNA fragment unpurified
2 : 500 bp DNA fragment purified

Fig. 1 Agarose gel electrophoresis of DNA fragment

2. Amplify 18 kb DNA fragment by PCR reaction and purify the DNA fragment by this kit. The result of agarose gel electrophoresis shows in Fig. 2. The recovery of DNA fragment is up to 70%.



1% Agarose gel electrophoresis

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

Fig. 2 Agarose gel electrophoresis of DNA fragment

VII. Cautions

1. It is recommended to elute DNA with sterilized water if the purified DNA is for sequencing.
2. It is recommended to elute DNA in Elution Buffer if the purified DNA is for long-time storage.
3. If the PCR products is amplified by RR902A and RR902Q kit, please do not use 9761 kit, otherwise the yield may be reduced.
4. The recovery rate of 20 - 50 kb DNA is low when using this kit.

VIII. Troubleshooting

Q1. How much does 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-heat 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 minimum amount of DNA is 500 ng. Please estimate the total amount of DNA sample before using the kit.

Q3. The yield of recovery DNA is low, why?
A3. The recovery efficiency of DNA is 70 - 95%. When the yield of recovery DNA is lower, consider the following aspects:
(1) Make sure that the volume of 100% ethanol specified on the bottle label has been added to Buffer WB.
(2) The centrifugation step before elution cannot be omitted. The centrifugation ensures that no residual ethanol will be carried over at the following elution step. The residual ethanol will affect the elution efficiency.
(3) Heat the Elution Buffer or sterile distilled water to 60℃ can improve elution efficiency.

Q4. The purified DNA have low biological activity.
A4. (1) There is residual salt ion in the eluted DNA. 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 the eluted DNA. Let it stand at room temperature for 2 minutes to help the residual ethanol to evaporate before adding the Elution Buffer in the Column.
(3) Use sterile distilled water to elute DNA.