Cat. # 9760

For Research Use

TaKaRa

TaKaRa MiniBEST Plasmid Purification Kit Ver.4.0

Product Manual

v201706Da

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I. Description

TaKaRa MiniBEST Plasmid Purification Kit Ver.4.0 is designed for rapid purification of plasmid DNA from a small amount of bacterial cultures. The kit is based on SDS-alkaline lysis of bacterial cells in combination with selective binding of plasmid DNA to the silica membrane of Spin Column. The protocol provides a simple method to achieve the rapid isolation of highly pure plasmid DNA and the whole procedure can be finished within 1 hour. 1 - 20 μg highly pure plasmid DNA (OD_{260}/OD_{280} = 1.8 - 2.0) can be obtained from 1 - 4 ml cultured bacterial cells. The procedure is unnecessary for the phenol extraction and ethanol precipitation. The purified plasmid DNA can be dissolved in Tris buffer or water and used directly for many downstream applications, such as DNA sequencing, transformation, *in vitro* transcription and restriction digestion and other enzymatic reactions.

II. Components (50 reactions)

This kit contains reagent set and column set.

Reagent Set

RNase A (10 mg/ml)	140 μl
Solution I	14 ml
Solution II*1	14 ml
Solution III*2	24 ml
Buffer WA*2	28 ml
Buffer WB*3	24 ml
Elution Buffer	2 mlx 2

*1 Contain alkali solution. Be careful to avoid contacting with skin and eyes. In the case of such contact, rinse immediately with plenty of water and seek medical advice.

*2 Contain strong denaturant. Be careful to avoid contacting with skin and eyes. In the case of such contact, rinse immediately with plenty of water and seek medical advice.

*3 Before the first use of the kit, add 56 ml of 100% ethanol to Buffer WB.

Column Set

Spin Column	50
Collection Tube	50

Reagents not supplied in this kit

1. 100% ethanol
2. Sterile purified water or Tris-HCl (pH 8.0)

III. Shipping and Storage

1. The kit can be stored at room temperature (15 - 25℃). If a precipitate is visible in some buffer, incubate the buffer at 37℃ for several minutes until the precipitate is dissolved completely.
2. RNase A can be stored at room temperature (15 - 25℃) stable for 6 months. It should be stored at -20℃ for long-term storage.
3. After adding RNase A to Solution I, it can be stored at 4℃ for 3 months.
4. The kit is shipped at room temperature (15 - 25℃).

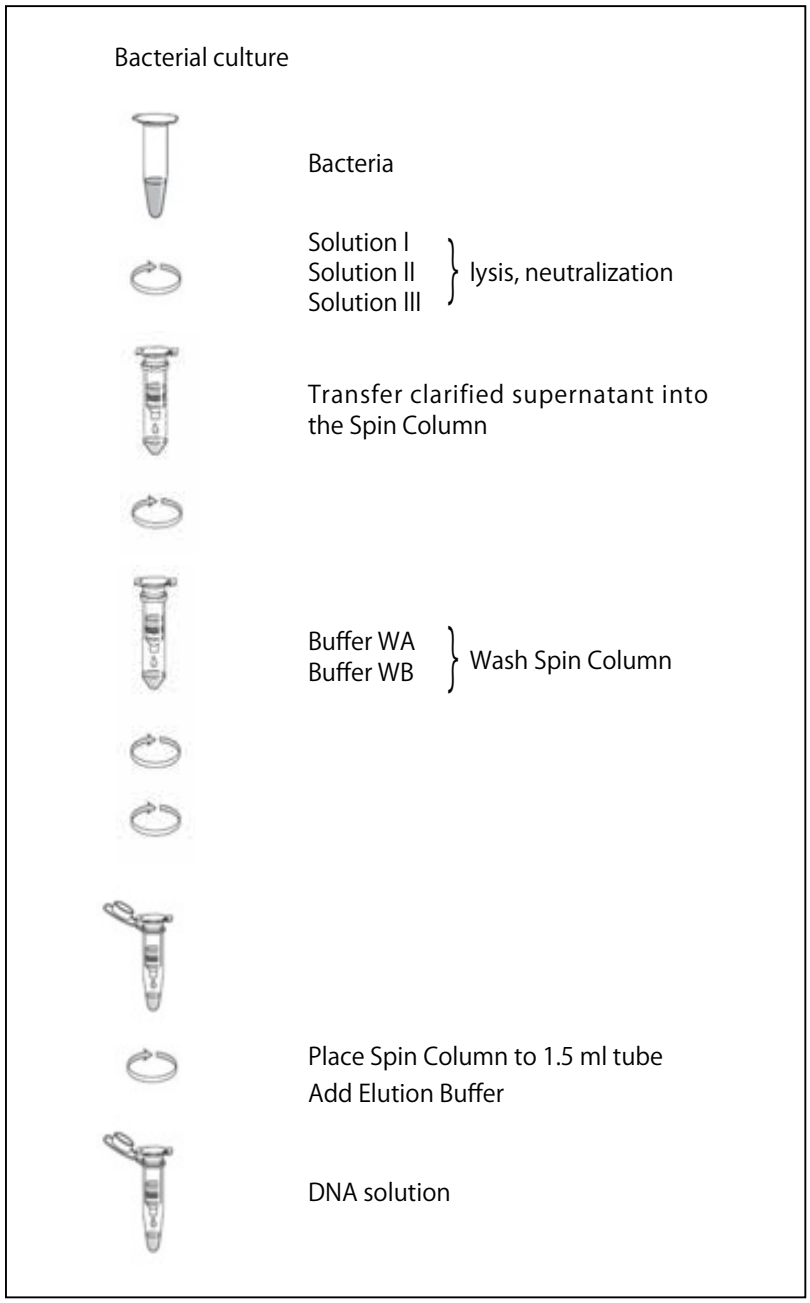
IV. Preparation before Use

1. Before the first use of the kit, transfer all RNase A solution to Solution I. Mix well and store at 4℃.
2. Before starting the kit, add 56 ml of 100% ethanol to Buffer WB.
3. Check if there's a precipitate in Solution II and Solution III. If a precipitate appears, warm at 37℃ for several minutes until the precipitate is dissolved completely. Do not shake Solution II vigorously otherwise a lot of bubbles will appear.
4. After using Solution II, close the buffer bottles quickly to avoid contact with air for a long time.
5. Precool Solution III at 4℃ before starting the experiment.
6. The reagents contain strong denaturant and alkali solution, please wear suitable protective clothing and gloves.

V. Protocol

Make sure RNase A has been added into Solution I and the indicated 100% ethanol has been added into Buffer WB.
Precool Solution III at 4℃ (or on ice) before experiment.
Protocol overview is below. The whole procedure can be accomplished in about 1 hour.

● Flow chart



● **Protocol in detail is as below**

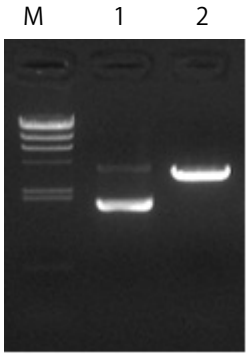
1. Growth of bacterial cultures.
   Pick a single colony from a freshly streaked selective plate into 1 - 4 ml of liquid culture containing the appropriate selective antibiotic. Incubate at 37℃ overnight with vigorous shaking. (Growth for 12 - 16 hours and do not more than 16 hours, otherwise the bacterial cells will be hard to lyse and the yield of plasmid DNA will be reduced.)
   Note) The culture volume should not be excessive because excessive bacteria will decrease lysis efficiency and result in poor purity of plasmid DNA.
2. Use 1 - 4 ml of the *E. coli* culture. Centrifuge at 12,000 rpm for 2 minutes to harvest the cell. Discard the supernatant.
3. Add 250 μl Solution I (containing RNase A). Resuspend the bacterial cell pellet completely by vortexing or pipetting up and down.
   Note) Be sure that the bacteria are completely resuspended by vortexing and no cell clumps remain before addition of Solution II.
4. Add 250 μl Solution II, and mix gently by inverting the tube 5 - 6 times to completely lysis the cell until the solution becomes viscous and slightly clear.
   Note) Do not allow the lysis reaction to proceed more than 5 minutes.
5. Add 350 μl of 4℃ precooling Solution III, and mix immediately and thoroughly by inverting the tube 5 - 6 times until a compact white pellet has been formed. Incubate at room temperature for 2 minutes.
6. Centrifuge at 12,000 rpm at room temperature for 10 minutes.
   Note) Centrifuging at 4℃ is not recommended for precipitation.
7. Place a Spin Column in a Collection Tube.
8. Apply the supernatant from Step 6 onto the Spin Column by decant or pipetting. Centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.
9. Pipette 500 μl of Buffer WA onto the Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
10. Pipette 700 μl of Buffer WB onto the Spin Column. Centrifuge at 12,000 rpm for 30 seconds. Discard the flow-through.
    Note) Make sure that the amount of 100% ethanol indicated on the bottle label has been added to Buffer WB.
11. Repeat Step 10.
12. Place the Spin Column back into the Collection Tube. Centrifuge at 12,000 rpm for an additional 1 minute to remove residual wash Buffer WB.
    Note) Residual ethanol from Buffer WB may inhibit subsequent enzymatic reaction.
13. Place the Spin Column in a new clean 1.5 ml tube.
    Add 50 μl Elution Buffer or sterile purified water to the center of the Spin Column membrane. Incubate for 1 minute at room temperature.
    Note) Pre-heat the Elution Buffer or sterile purified water to 60℃ will improve the elution efficiency.
14. Centrifuge at 12,000 rpm for 1 minute to elute DNA.

If the laboratory have negative pressure device suitable for Spin Column interface, operate following protocol after Step 6 above-mentioned.

7. Attach the vacuum manifold to the vacuum source. Insert a Spin Column into one of the complimentary fittings.
8. Transfer the supernatant from Step 6 into the Spin Column. Switch on vacuum source and adjust the negative pressure (flow rate controlled at 1 drop/second). Continue to apply the vacuum slowly until no liquid remains in the column. Switch off vacuum source.
9. Adjust the regulator of negative pressure device to the maximum. Pipette 500 μl of Buffer WA into Spin Column. Switch on vacuum source until no fluid remains in the Spin Column. Switch off vacuum source.
10. Pipette 700 μl of Buffer WB into Spin Column. Switch on vacuum source until no fluid remains in the Spin Column. Switch off vacuum source.
    Note) Make sure that the amount of 100% ethanol indicated on the bottle label has been added to Buffer WB.
11. Repeat Step 10. Remove the Spin Column from negative pressure device and place to the Collection Tube.
12. Centrifuge at 12,000 rpm for 1 minute.
13. Place the Spin Column to a new clean 1.5 ml tube. Add 50 μl Elution Buffer or sterile purified water to the center of the Spin Column membrane. Incubate for 1 minute at room temperature.
    Note) Pre-heat the Elution Buffer or sterile purified water to 60℃ will improve the elution efficiency.
14. Centrifuge at 12,000 rpm for 1 minute to elute the DNA.

VI. Experimental Example

About 16 μg of plasmid DNA ($OD_{260}/OD_{280} \geqq 1.8$) is purified from 1.5 ml of bacterial culture (JM109) grown in TB broth. The plasmid is pUC119. Digest the plasmid by *Hin*d lll for 1 hour. (electrophoresis result is shown in Figure 1).



1% Agarose gel electrophoresis
M : λ *Hin*d lll digest
1 : Plasmid DNA
2 : Plasmid DNA (digested by *Hin*d lll)

Figure 1. Electrophoresis of plasmid DNA

VII. Cautions

1. The volume of starting material should be controlled within 1 - 4 ml. Excessive bacteria will cause incomplete lysis of bacteria and reduce the purity and yield of plasmid DNA.
2. After adding each Solution II and Solution III into the mixture, do not mix vigorously. Vigorous shaking or vortexing will cause contamination with bacterial genomic DNA.
3. After adding Solution III, mix thoroughly until the protein and genomic DNA form a white precipitate. Then centrifuge and make the precipitate assemble in the bottom of the tube.

   If there are still some precipitates suspending in the solution, invert several times and centrifuge at high speed for 3 - 5 minutes.
4. Elute the plasmid DNA with sterile purified water if the purified plasmid DNA is used for DNA sequencing.
5. The plasmid DNA should be eluted with Elution Buffer for long time storage.

VIII. Troubleshooting

Q1. How much cell culture is appropriate?

A1. For the high-copy plasmid (such as pUC118) for example, yield of purified plasmid DNA from 2 ml or 4 ml of bacterial culture are 10 - 15 μg of plasmid DNA. Generally, we recommend 2 ml of bacterial culture for plasmid DNA extraction from high-copy plasmid.

Q2. How about the extraction of low-copy plasmid using the kit?

A2. For low-copy plasmid, it is recommend to extract plasmid DNA from 4 ml of bacterial culture. Taking pBI121 for example, 2 - 3 μg of highly-purified plasmid DNA can be extracted from 4 ml of bacterial culture.

Q3. Why is there low recovery of the plasmid DNA?

A3. Usually, 10 μg of high-purified plasmid can be extracted from 2 ml of pUC118/JM109 in LB culture grown overnight. When there is low recovery of plasmid DNA, the following aspects can be considered:

(1) The bacterial cell is not fresh (long-term storage low temperatures). Please spread the culture on a plate and pick a new colony into liquid culture to recultivate.
(2) The copy of plasmid is too low. When use a low-copy plasmid, the yield of plasmid DNA is low, please increase the volume of bacterial culture.
(3) Confirm the accuracy of operation. Strictly follow the protocol.
(4) Pre-heat the Elution Buffer or sterile purified water at 60℃ will improve the elution efficiency.

Q4. Why is the solution not clarified after adding Solution II?

A4. (1) Excessive bacteria will lead to incomplete lysis of bacteria. The volume of bacterial culture used each time is 1 - 4 ml.
(2) Bacteria has not resuspension completely after adding Solution I. Be sure that the bacteria are completely resuspended by vortexing and no cell clumps remain before addition of Solution II.

Q5. Why is the sequencing result of plasmid DNA not good?

A5. (1) The amount of template DNA added is not accurate. Perform quantitative analysis to the plasmid DNA correctly. When using absorbance to quantitate the plasmid DNA, sometimes the impurity in DNA solution will affect the evaluation of absorbance. Therefore it is recommended to apply agarose gel electrophoresis to quantitate the plasmid DNA.
(2) The purity of plasmid DNA is not good. Please follow the protocol strictly and use fresh bacterial culture to extract plasmid.
(3) Use sterile purified water to elute the DNA.
(4) The configuration of insert DNA is complicated. Some DNA has complicated configuration (such as GC rich, repetitive sequence) so that it is hard for sequencing, and then the sequencing method should be improved.

Q6. How much is the minimum elution volume of plasmid DNA?

A6. We recommend the elution volume is 30 - 100 μl which have the highest yield (Note: Load elution buffer onto the center of the silica membrane). The yield will be poor when the elution volume is less than 30 μl. The elution volume may be reduced down to 20 μl if high concentration plasmid DNA is required, but the yield of plasmid will be decrease slightly. Use pre-heating eluate, and incubate for at least 1 minute at room temperature before centrifuge in order to increase the elution efficiency.

Q7. Why does the purified plasmid DNA contaminate with genomic DNA?

A7. (1) Vortex or mix Cell lysate gently after addition of Solution II, do not vortex vigorously.
(2) Do not allow the lysis reaction to proceed more than 5 minutes after addition of Solution II.
(3) Do not incubate cultures for more than 16 hours. The cultivation of 12 - 16 hours is better.

Q8. Why does the purified plasmid DNA contaminate with RNA?

A8. (1) Make sure that RNase A has been added to Solution I.
(2) Solution I after addition of RNase A should store at 4℃. Add RNase A to Solution I again if the storage time exceed 3 months because the activity of RNase A (Cat. #2158) has been decreased.