PRODUCT INFORMATION

BP-10 SPIN COLUMN PLASMID DNA MINIPREPS KIT

Components	PMIP-100 , 100 Preps	PMIP-200 , 200 Preps
RNase A(10mg/ml)	**240µl**	**2x240µl**
Blue indicator	**240ul**	**2x240ul**
Solution I	**12ml**	**2x12ml**
Solution II	**22ml**	**2x22ml**
Solution III	**40ml**	**2x40ml**
Wash Solution	**24ml**	**2x24ml**
Elution Buffer	**10ml**	**2x10ml**
BP-10 Column	**100**	**2x100**
2.0 ml Collection Tube	**100**	**2x100**
Protocol	**1**	**1**

A) Before use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase should be stored at 4ºC for frequent use and at -20ºC for infrequent use.
B) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37ºC.
C) Before use, add 96ml of 96-100% ethanol to 24ml Wash Solution for PMIP-100. For other volumes of wash solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).
D) Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower.

Storage: With the exception of the RNase A, the kit may be stored at room temperature. The RNase A should be stored at 4ºC. The kit is stable for 12 months at room temperature. For longer storage, keep all contents cold.

Principle:

This kit provides a simple and efficient method for Mini plasmid DNA purification. The plasmid DNA is selectively adsorbed in silica gel-based BP-10 column and other impurities such as proteins, salts, nucleotides, oligos (<40-mer) are washed away. The plasmid DNA is then eluted off the column and can be used for any downstream application.

Applications:

- Plasmid DNA purification

Features:

- Fast. Entire procedure takes 15-20 minutes.
- Preparation of high quality plasmid DNA from bacterial cultures. Purified DNA can be used in any downstream applications such as sequencing, PCR, transformation, restriction enzymatic digestion, and ligation.
- High yields (>80%)
- No phenol / chloroform extraction or ethanol precipitation is required.

Protocol for Purification of Plasmid DNA

1. Add 1.5ml overnight culture to a 1.5ml microfuge tube and centrifuge at 8,000rpm for 1 minute. Drain the liquid completely. For low copy number of plasmid, see the protocol on the following page.
2. Optional, add blue indicator to solution I at ratio (V/V) 1:50.
3. Add 100ul of Solution I to the pellet, mix gently， and keep for 2 minutes.
4. Add 200ul of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute. **To prevent contamination from genomic DNA, do not vortex.** If the blue indicator is added into solution I, the color of the mixture should turn blue after addition of solution II
5. Add 350ul of Solution III, and mix gently. Incubate at room temperature for 2 minute. If the blue indicator is used, the color of the mixture should turn into colorless after addition of solution III.
6. Centrifuge at 14,000rpm for 5 minutes.
7. Transfer the above supernatant (step 5) to the BP-10 column. Centrifuge at 10,000rpm for 30 seconds.
8. Discard the flow-through in the tube. Add 500ul of Wash Solution to the column, and centrifuge at 10,000rpm for 1 minute.
9. Repeat wash procedure in step 8.
10. Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional 1 minute to remove any residual Wash Solution.
11. Transfer the column to a clean 1.5ml microfuge tube. Add 50ul of Elution Buffer into the center part of the column and incubate at room temperature for 1-2 minutes. Centrifuge at 10,000 rpm for 1 minutes.
12. Store the purified DNA at -20ºC.

Note: It is extremely important to add the Elution Buffer into the center part of the column. Incubating the column with the Elution Buffer at higher temperatures (37 to 50ºC) may slightly increase the yield especially of large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55 to 80ºC may also slightly increase elution efficiency.

Protocol for Purification of Low Copy Plasmid DNA

1. Use 3-5ml overnight culture. Add 1.5ml of culture to a 1.5ml microfuge tube and centrifuge at 12,000rpm for 1 minute. Drain the liquid completely and repeat with another portion of culture (in the same tube).
2. Optional, add blue indicator to solution I at ratio (V/V) 1:50.
3. Add 200ul of Solution I to the pellet, mix gently and keep for 2 minutes. If the blue indicator is added into solution I, the color of the mixture should turn blue after addition of solution II.
4. **Add 400ul of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at room temperature for 1 minute. To prevent contamination from genomic DNA, do not vortex.** If the blue indicator is used, the color of the mixture should turn into colorless after addition of solution III.
5. Add 700ul of Solution III, and mix gently. Incubate at room temperature for 2 minute.
6. Centrifuge at 10,000 rpm for 5 minutes.
7. Transfer half of the above supernatant (step 6) to the BP-10 column. Centrifuge at 10,000 rpm for 1 minute. Discard the flow-through in the tube and add the second half of the mix, Centrifuge again at 5,000rpm for 1 minute.
8. Discard the flow-through in the tube. Add 500ul of Wash Solution to the column, and centrifuge at 10,000rpm for 1 minute.
9. Repeat wash procedure in step 8 (optional).

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10. Discard the flow-through in the collection tube. Centrifuge at 10,000 rpm for an additional 1 minute to remove any residual Wash Solution.
11. Transfer the column to a clean 1.5ml microfuge tube. Add 50ul of Elution Buffer into the center part of the column and incubate at room temperature for 1-2 minutes. Centrifuge at 10,000 rpm for 1 minutes.
12. Store the purified DNA at –20ºC.

Note: It is extremely important to add the Elution Buffer into the center part of the column. For higher concentration of DNA, two elution steps may be carried out with 25ul of Elution Buffer each, rather than two 50ul steps. Incubating the column with the Elution Buffer at higher temperatures (37 to 50ºC) may slightly increase the yield especially of large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55 to 80ºC may also slightly increase elution efficiency.

For research only not for diagnostic and clinic use

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