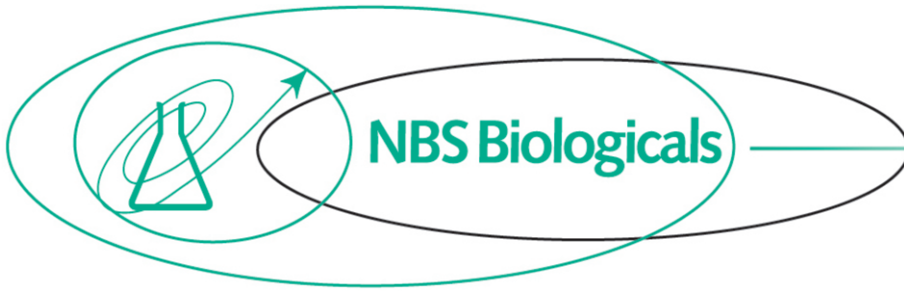
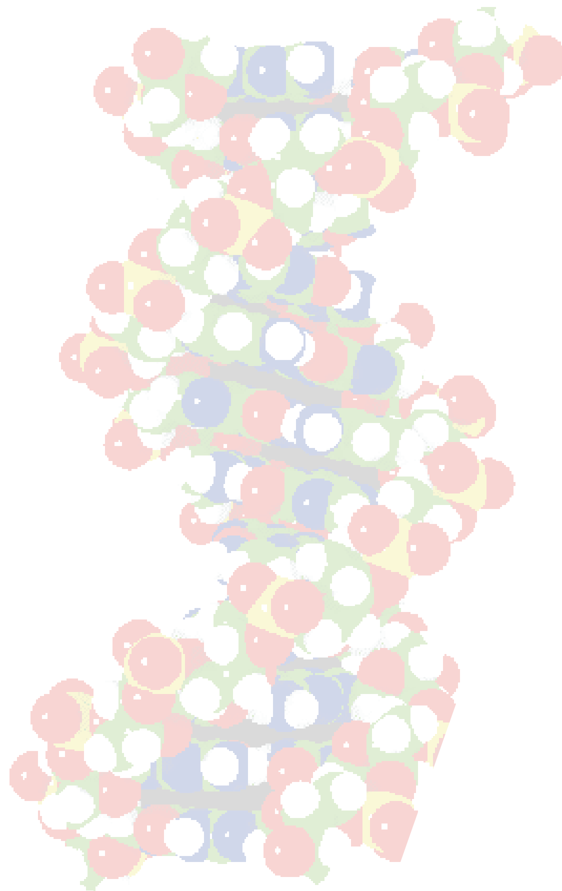


NBS

DNA Purification

Spin Column Purification
Plasmid DNA Handbook



- *Spin Column* Plasmid Miniprep Kit
- *Spin Column* Plasmid Mediprep Kit
- *Spin Column* Plasmid Maxiprep Ki

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Introduction:

The Spin Column Kits provide a simple and efficient method for purification of plasmid DNA. The DNA is selectively adsorbed in silica gel-based column and other components are washed away. The DNA is then eluted off the column and can be used for any downstream applications.

The purification method used in these protocols does not require use of phenol, chloroform, or CsCl. The DNA is purified without an additional step of ethanol precipitation.

Limitations of Use:

These kits are designed for research only. The purified plasmid DNA should not be used for live animal transfections. It is also not to be used for human diagnostic or drug production purposes.

Features:

Simple, Fast and Efficient
Preparation of high quality DNA which can be used in any downstream applications such as sequencing, transformation and restriction digestions
High yield and Reproducible
No phenol / chloroform extraction; No CsCl centrifugation; No ethanol precipitation.

Applications:

Spin Column Plasmid DNA Miniprep Kit NBS-413, NBS-414, NBS-614
This kit can be used for purification of plasmid DNA from 40bp-40kb.
Spin Column Plasmid Mediprep Kit NBS-463, NBS-464
Purification of up to 200ug of plasmid DNA.
Spin Column Plasmid Maxiprep Kit NBS-465, NBS-466
Purification of up to 500ug of plasmid DNA.

Storage:

RNase A should be stored at 4°C. The other Plasmid DNA Spin Kits components can be stored dry at room temperature (15°C-25°C). Kits can be stored for up to 18 months without showing any reduction in performance and quality. RNase A stock solution can be stored for 2 years at 4°C. After addition of RNase A, Solution I is stable for 6 months at 4°C. Binding Buffer II from Spin Column DNA Gel Extraction Kit may form a precipitate after storing for longer than a year. The precipitate should not affect performance and results of the kit.

Quality Control:

Each lot of Spin Column kit is tested against predetermined specifications to ensure consistent product quality.

Protocol: *Spin Column* Plasmid Miniprep Kit NBS-413, NBS-414, NBS-614:

Spin Column Plasmid DNA Minipreps Kit Components	NBS-413 50 Preps	NBS-414 100 Preps	NBS-614 250 Preps
Solution I	6ml	12ml	30ml
Solution II	12ml	24ml	2X30ml
Solution III	25ml	2X25ml	5X25ml
Wash Solution	12ml	24ml	2X30ml
Elution Buffer	5ml	10ml	25ml
RNase A Solution (10mg/ml)	120ul	240ul	600ul
Spin Column	50	100	250
2.0 ml Collection Tube	50	100	250
Protocol	1	1	1

a. Before use, add the RNase A Solution to the bottle containing Solution I and mix well. Solution I with RNase A should be stored at 4ºC for frequent use and at -20ºC for in frequent 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 48ml of 96-100% of ethanol to 12ml Wash Solution for NBS-413; add 96ml of 96-100% ethanol to 24ml Wash Solution for NBS-414; add 120ml of 96-100% ethanol to 30ml Wash Solution for NBS-614. 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.

Principle:

This kit provides a simple and efficient method for Mini plasmid DNA purification. The plasmid DNA is selectively adsorbed in silica gel-based spin 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.

Protocol for Purification of Plasmid DNA:

1. Add 1.5ml overnight culture to a 1.5ml microfuge tube and centrifuge at 12,000rpm for 2 minutes. Drain the liquid completely. For low copy number of plasmid, see the protocol on the following page.
2. Add 100ul of Solution I to the pellet, mix well, and keep for 1 minute.
3. Add 200ul of Solution II to the mixture, and 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.
4. Add 350ul of Solution III, and mix gently. Incubate at room temperature for 1 minute.
5. Centrifuge at 12,000rpm for 5 minutes.
6. Transfer the above supernatant (step 5) to the spin column. Centrifuge at 10,000rpm for 2 minutes.
7. Discard the flow-through in the tube. Add 500ul of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Centrifuge at 10,000rpm for an additional 1 minute to remove any residual Wash Solution.
10. 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 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.
11. 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 temperature (37ºC to 50ºC) may slightly increase the yield especially of large (>10,000bp) DNA Plasmids. Prewarming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

Protocol for Purificatoin of Low Copy Plasmid DNA:

1. Use 3-5ml overnight culture. Add overnight culture to a 1.5ml microfuge tube and centrifuge at 12,000rpm for 2 minutes. Drain the liquid completely and repeat with another portion of culture (in the same tube).
2. Add 200ul of Solution I to the pellet, mix well and keep for 1 minute.
3. Add 400ul of Solution II to the mixture, and 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.
4. Add 700ul of Solution III, and mix gently. Incubate at room temperature for 1 minute.
5. Centrifuge at 12,000 rpm for 5 minutes.
6. Transfer half of the above supernatant (step 5) to the column. Let the column stand for 2min. Centrifuge at 10,000 rpm for 2 minutes. Discard the flow-through in the tube and add the second half of the supernatant, centrifuge again at 10,000 rpm for 2 minutes.
7. Discard the flow-through in the tube. Add 500ul of Wash Solution to the column, and centrifuge at 10,000rpm for 2 minutes.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Centrifuge at 10,000 rpm for an additional 1 minute to remove any residual Wash Solution.
10. 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 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.
11. Store the purified DNA at –20ºC.

Troubleshooting Guide: *Spin Column* Plasmid Minipreps Kit:

- Low Yield

  There are a number of variables that can cause low yield.

  a. Each of the steps has to be strictly followed.
  b. Make sure there is no precipitant in Solution I, II or III. If precipitant is present in the buffer, heat up the solution to 37°C and shake well.
  c. Low culture density. Make sure that the temperature in the incubator is stable and shaking speed provides sufficient aeration of the culture.
  d. Very high cell density, therefore incomplete cell lyses. Double the volume of Solution I, II and III.

- Contamination of chromosomal DNA
Do not vortex the sample after adding solution II and III. Vigorous shaking will cause shearing of chromosomal DNA. Smaller pieces of chromosomal DNA will be captured on silica gel and carried over with purified plasmid DNA.

- RNA contamination
RNase activity is weaked or lost. Add addition RNAse A to Solution I and store at 4°C.

- OD 260nm/280nm ratio outside 1.9-2.2 range
If the ratio of OD260nm/280nm is greater than 2.2, there may be traces of ethanol present.
If the ratio of OD260nm/280nm is smaller than 1.9, there is chance of protein contamination. Make sure the sample is mixed well after Solution III is added and after spinning down.

Protocol: *Spin Column* Plasmid Mediprep Kit NBS-463, NBS-464:

Column Plasmid Mediprep Kit Components	NBS-463 10 Preps	NBS-464 20 Preps
Solution I	98ml	196ml
Solution II	200ml	2x200ml
Solution III	2x175ml	4x175ml
Wash Solution	24ml	48ml
Elution Buffer	20ml	40ml
RNase A (10mg/ml)	2ml	2x2ml
Spin Column	10	20
50ml Collection Tube	10	20
Protocol	1	1

(a) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37°C.
(b) Before use, add 96 ml of 100% of ethanol to 24 ml Wash Solution for NBS-463, add 192 ml of 100% ethanol to 48ml Wash Solution for NBS-464. 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).
(c) Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 20% lower.
(d) Before use, add RNase A to Solution I. Solution I should be stored at 4°C for frequent use ,or stored at -20 °C for longer period use.

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 purification of up to 200ug of plasmid DNA. Bacterial cultures are lysed and the lysates are cleared by routine methods (Solution I, II, III). Plasmid DNA from the cleared lysates is selectively adsorbed in spin column and other impurities such as proteins, salts, oligos (<40-mer) and nucleotides are washed away. Pure DNA is eluted in low-salt buffer or water. Purified Plasmid DNA can be used for any downstream applications such as sequencing, restriction reactions, labeling, transformation, PCR and Southern-blotting.

Protocol for Purification of Plasmid DNA:

1. Add 200ml overnight culture to a appropriate centrifuge tube and centrifuge at 6,000 rpm for 10 minutes. Drain the liquid completely.
2. Add 8 ml of Solution I to the pellet. Mix gently and keep for 2 minute.
3. Add 16 ml of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at RT for 2 minutes.

Note: To prevent contamination from genomic DNA, do not vortex.

4. Add 28 ml of Solution III, and mix gently. Incubate at RT for 2 minutes.
5. Spin at 5000Xg for 10 minutes.
6. Place spin column into a 50 ml collection tube. Transfer the above supernatant (step 5) to the column, Stand at RT for 5 minutes. Spin at 4000Xg for 3-5 minutes.

Notes: The column cannot hold entire supernatant. Please apply in multiple times. Please spin briefly (1-2 min) at 4000Xg between each application. After the final application of entire content in the column, please spin at 4000Xg for 3-5 min.

7. Discard the flow-through in the tube. Add 5 ml of Wash Solution to the column, and spin at 4000Xg for 3-5 minutes.
8. Repeat wash procedure in step 7.
9. Discard the flow-through in the collection tube. Spin at 4000Xg for additional 5 minutes to remove residual Wash Solution.
10. Transfer the column to a clean pre-warmed 50 ml centrifuge tube. Add 1ml of Elution Buffer into the center part of the membrane of the column and incubate at 37 - 50 ^{o}C for 2 minutes. Spin at 4000Xg for 2 minutes.
11. Add additional 1 ml of Elution Buffer into center of spin column and spin at 4000Xg for 2 minutes. Purified plasmid DNA can immediately be used or be stored at –20°C freezer.

Note: It is important to add the Elution Buffer into the center part of spin column. Elution Buffer at 55-80 ^{o}C could increase elution efficiency. Two times elution is recommended.

Protocol: *Spin Column* Plasmid Maxiprep Kit NBS-465, NBS-466:

Spin Column Plasmid Mediprep Kit Components	NBS-465 10 Preps	NBS-466 20 Preps
Solution I	245ml	2x245ml
Solution II	2x250ml	4x250ml
Solution III	4x225ml	8x225ml
Wash Solution	24ml	48ml
Elution Buffer	50ml	100ml
RNase A (10mg/ml)	5ml	10ml
Spin Column	10	20
50ml Collection tube	10	20
Protocol	1	1

a) Solution II may form a precipitate upon storage. If necessary, dissolve the precipitate by warming at 37°C.
b) Before use, add 96 ml of 100% of ethanol to 24 ml Wash Solution for NBS-465, add 192 ml of 100% ethanol to 48ml Wash Solution for NBS-466. 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).
c) Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 20% lower.
d) Before use, add RNase A to Solution I. Then Solution I should be stored at 4 °C for frequent use, or stored at -20 °C if not use for a long period.

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 purification of up to 500 ug of plasmid DNA. Bacterial cultures are lysed and the lysates are cleared by routine methods (Solution I, II, III). Plasmid DNA from the cleared lysate are selectively adsorbed in spin column and other impurities such as proteins, salts, oligos (<40-mer) and nucleotides are washed away. Pure DNA is eluted in low-salt buffer or water.
Purified plasmid DNA can be used for any downstream applications such as sequencing, restriction reactions, labeling, transformation, PCR and Southern-blotting.

Protocol for purification of Plasmid DNAI:

1. Add 500 ml overnight culture to an appropriate centrifuge tube and centrifuge at 6,000 rpm for 10 minutes. Drain the liquid completely.
2. Add 20 ml of Solution I to the pellet, mix gently and keep on ice for 2 minute.
3. Add 40 ml of Solution II to the mixture, mix gently by inverting the tube 4-6 times and then keep at RT for 2 minutes.

Note: To prevent contamination from genomic DNA, do not vortex.

4. Add 70 ml of Solution III, and mix gently. Incubate at RT for 2 minutes.
5. Spin at 5500Xg for 10 minutes.
6. Place column into a 50 ml collection tube. Transfer the above supernatant (step 5) to the column. Stand at RT for 5 minutes. Spin at 4000Xg for 3-5 minutes.

Notes: The column cannot hold entire supernatant. Please apply in multiple times. Please spin briefly (1-2 min) at 4000Xg between each application. After the final application of entire content in the column, please spin at 4000Xg for 3-5 min.

7. Discard the flow-through in the tube.
8. Repeat step 6-7 until all supernatant from step 5 has been applied onto the column.
9. Add 5 ml of Wash Solution to the column, and spin at 4000Xg for 3-5 minutes.
10. Repeat wash procedure in step 9.
11. Discard the flow-through in the collection tube. Spin at 4000Xg for additional 5 minutes to remove residual Wash Solution.
12. Transfer the column to a clean 50 ml microfuge tube. Add 2.5ml of Elution Buffer into the center part of the membrane of the column and incubate at 37 - 50°C for 2 minutes. Spin at 4000Xg for 2 minutes. Store DNA at –20°C freezer. For higher recovery yield additional 2.5ml of Elution Buffer is added to the center part of the column and spin at 8000 rpm for 2 minutes. Measure OD260. Purified plasmid DNA is ready to use, or store at freezer for long period use.

Note: It is important to add the Elution Buffer into the center part. Pre-warm Elution Buffer at ·80 °C could increase elution efficiency. Two times elution is recommended.

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