NBS Biologicals

NBS

DNA Purification

Spin Column Purification
DNA Cleanup Handbook

- *Spin Column* PCR Purification Kit
- *Spin Column* Gel Extraction Kit
- *Spin Column* Reaction Cleanup Kit

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Introduction:

The Spin Column Kits provide a simple and efficient method for extraction of DNA from agarose gels, and purification of DNA from enzymatic reactions such as PCR or restriction enzyme digestions.

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, PCR, transformation or restriction digestions
High yield and Reproducible
High Capacity. Up to 10ug of DNA per column.

Applications:

Spin Column PCR Purification Kit NBS-363, NBS-364, NBS-664
Recovery of 40bp-40kb DNA fragments from reaction solutions.
Spin Column DNA Gel Extraction Kit NBS-353, NBS-354, NBS-654
Recovery of 40bp-40kb DNA fragments from agarose gels.
Spin Column Reaction Cleanup Kit NBS-367, NBS-368, NBS-668
Purification of DNA fragments form enzymatic reactions.

Storage:

The Spin Column Kits should be stored dry at room temperature (15^{o}C-25^{o}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^{o}C. After addition of RNase A, Solution I is stable for 6 months at 4^{o}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* PCR Purification Kit NBS-363, NBS-364, NBS-664:

Prod	Spin Column PCR Purification Kit Components	NBS-363 50 Preps	NBS-364 100 Preps	NBS-664 250 Preps
	Binding Buffer I	20ml	2x20ml	4x25ml
	Wash Solution [A]	20ml	2X20ml	2X40ml
	Elution Buffer[B]	5ml	10ml	25ml
	Spin Column	50	100	250
	1.0 ml Collection Tube	50	100	250
	Protocol	1	1	1

a. Before use, add 80ml of 96-100% of ethanol to 20ml Wash Solution for NBS-363; add 160ml of 96-100% ethanol to 40ml Wash Solution for NBS-364; add 320ml of 96-100% ethanol to 80ml Wash Solution for NBS-664. 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).

b. Elution Buffer is 2mM 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:

The spin column purification kits utilise a silica-gel membrane that selectively absorbs up to 10ug of DNA fragments in the presence of specialised binding buffers. Nucleotides, oligos (<40-mer), enzymes, mineral oil and other impurities do not bind to the membrane and are washed away. The DNA fragments can then be eluted off the column in small volume and used in downstream applications without further processing.

Protocol for Purification of PCR Products:

1 Transfer PCR reaction mixture to a 1.5ml microfuge tube and add 3 volumes of Binding Buffer I.

2 Transfer the above mixture solution to the spin column and let it stand at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes.

3 Remove the flow-through in the tube. Add 750ul of Wash Solution to the column and centrifuge at 10,000 rpm for 2 minutes.

4 Repeat washing procedure in step 3. Spin at 10,000 rpm for an additional minute to remove any residual Wash Solution.

5 Transfer the column into a clean 1.5ml microfuge tube and add 30-50ul of Elution Buffer. Incubate at room temperature for 2 minutes. Centrifuge at 10,000 rpm for 2 minutes to elute the DNA.
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.

6 Store the purified DNA at -20ºC.
Note: 1; If PCR reaction mixture contains seriously non-specific amplified DNA fragments, use of the DNA Gel Extraction Kit is recommended.
2; This kit can not remove the template and primers with chain length longer than 50-mer.

Protocol: *Spin Column* Gel Extraction Kit NBS-353, NBS-354, NBS-654:

Spin Column Gel Extraction Kit Components	NBS-353 50 Preps	NBS-354 100 Preps	NBS-654 250 Preps
Binding Buffer II	50ml	2X50ml	5X50ml
Wash Solution	20ml	2X20ml	2X40ml
Elution Buffer	5ml	10ml	25ml
Spin Column	50	100	250
2.0ml Collection tube	50	100	250
Protocol	1	1	1

a. Before use, add 80ml of 96-100% of ethanol to 20ml Wash Solution for NBS-353; add 160ml of 96-100% ethanol to 40ml Wash Solution for NBS-354; add 320ml of 96-100% ethanol to 80ml Wash Solution for NBS-654. 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).

b. Elution Buffer is 2mM 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.

Note: The kit is observed to have better performance when TAE, rather than TBE, is used.

Principle:

The spin column purification kit utilises a silica-gel based membrane which selectively adsorbs up to 10ug of DNA fragments in the presence of specialised binding buffers. Nucleotides, oligos (<40- mer), enzymes, mineral oil and other impurities do not bind to the membrane and are washed away. DNA fragments are then eluted off the column and can be used for downstream protocols without further processing.

Protocol for Agarose Gel:

1 Excise the DNA fragment from the gel with a clean, sharp scalpel. Weigh the gel slice and transfer to a 1.5mL microfuge tube.

2 Add 400ul of Binding Buffer II for each 100mg of gel weight (for example, a gel slice weighing 125mg would require 500ul of Binding Buffer II). Incubate at 50^0C -60^0C for 10 minutes and shake occasionally until agarose is completely dissolved. For high concentration gels (1.5-2.0%), 700ul of Binding Buffer II per 100mg of agarose gel are added.

3 Add the above mixture to the spincolumn and let stand for 2 minutes. Centrifuge at 10,000rpm for 2 minutes and discard the flow-through in the tube.
4 Add 750ul of Wash Solution, and centrifuge at 10,000rpm for one minute. Discard the solution in the tube.
5 Repeat step 4. Centrifuge at 10,000rpm for an additional 1-minute to remove any residual Wash Buffer.
6 Place the column in a clean 1.5ml microfuge tube. Add 30-50ul of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes. Centrifuge at 10,000rpm for 2 minutes to elute DNA.

Note: It is extremely important to add the Elution Buffer to the center of the column. Incubating the column at higher temperature (37ºC to 50ºC) may slightly increase the yield. Pre-warming the Elution Buffer at 55ºC to 80ºC may also slightly increase elution efficiency.

7 Store the purified DNA at -20ºC.

Protocol for DNA purification from enzymatic reactions:

1. Transfer entire contents of the reaction mixture to a 1.5ml microfuge tube and add 3 volumes of Binding Buffer II. Mix by inverting the tube a few times.
2. Add the above mixture to the spincolumn and let the column stand for 2 minutes. Centrifuge at **10,000rpm** for 1 minute and discard the flow-through in the tube.
3. Add 750ul of Wash Solution, and centrifuge at 10,000rmp for 1 minute. Discard the solution in the tube.
4. Repeat step 3. Spin at 10, 000rpm for an additional minute to remove any residual Wash buffer.
5. Place the column in a clean 1.5ml microfuge tube. Add 30-50ul of Elution Buffer to the center of the column and incubate at room temperature for 2 minutes.

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.

6. Centrifuge at 10,000rpm for 2 minutes to elute the DNA.
7. Store the purified DNA at -20ºC.

Troubleshooting Guide: *Spin Column* PCR Purification Kit and Gel Extraction Kit:

Low Yield

There are number of variables that can cause low yield

a. Each step has to be strictly followed.
b. Make sure column binding capacity 10ug is not exceeded.

Samples floats upon loading in agarose gel

The sample contains ethanol from washing step. Discard the liquid waste from the collection tube after washing step, and spin again for additional two minutes before the final elution step.

Protocol: *Spin Column* Reaction Cleanup Kit NBS-367, NBS-368, NBS-668:

Spin Column Reaction Cleanup Kit Components	NBS-367 50 Preps	NBS-368 100 Preps	NBS-668 250 Preps
Cleanup Solution	20ml	40ml	100ml
Wash Solution	12ml	24ml	2X30ml
Elution Buffer	5ml	10ml	25ml
Spin Column	50	100	250
2.0ml Collection tube	50	100	250
Protocol	1	1	1

a. Before use, add 48ml of 100% of ethanol to 12ml Wash Solution for NBS-367, or add 96ml of 100% ethanol to 24ml Wash Solution for NBS-368, add 60ml of 100% ethanol to 30ml Wash Solution for NBS-668. 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).

b. Elution Buffer is 2mM 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: The kit is stable for 12 months at room temperature. For longer storage, keep all contents of the kit cold.

Principle:

This DNA Reaction Cleanup kit provides a simple, efficient method for purification of DNA fragments from variable enzymatic reactions such as cDNA synthesis, ligation, restriction digestions, tailing, PCR, alkaline phosphatase, nick translation, due terminators products from PCR reaction mixture. It is also an ideal tool to desalt the solution of DNA as well as to remove residual organic solvents or unincorporated nucleotides of promoters (<40-mer) from reaction mixture. The kit utilises a technology which adsorbs selectively up to 10ug DNA fragments in the column in the presence of specialised binding buffers. Nucleotides, enzymes, mineral oil and other impurities do not bind the column in the plate. DNA fragments can be eluted readily with elution buffer.

Application:

- DNA Cleanup from the enzymatic reactions
- Removal of nucleotides and primers (<40-mer)

Features:

- Rapid and economical. Entire procedure takes 40 minutes.
- High yields (60-80%). It is suitable to recover 100bp-40kb DNA fragments.
- Efficient removal of contaminants. Purified DNA can be used in any downstream applications such as sequencing, labelling, restriction enzymatic digestions, ligations or transformations.
- No phenol/chloroform extraction or ethanol precipitation.

Procedure for Purification of DNA Products:

1. Transfer DNA mixture to a 1.5 ml microfuge tube and add 3 volumes of Cleanup Solution.
2. Place a column into a 2.0 ml collection tube. Transfer the above mixture solution to the column, and
let the column stand at room temperature for 2 minutes. Spin at 5000 rpm for 1 minute.
3. Discard flow-through. Add 500 ul Wash Solution to the column and spin at 8,000 rpm for 1 minute.
Discard flow-through and place column back to the same collection tube.
4. Add 500 ul Wash Solution to the column, spin at 8,000 rpm for 1 minute. Discard flow-through and
spin once more to remove residue of Wash Solution.
5. Transfer column to a clean 1.5 ml microtube. Add 30-50ul Elution Buffer or water onto the center part
of the column, incubate at 50oC for 2 minutes. Spin down at 10,000 rpm for 1 minute. Purified DNA
including PCR* product is ready for use or kept at – 20oC.

Note:

1. Incubation at 37-50 oC can improve recovery yield.
2. If PCR* reaction mixture contain seriously non-specific amplified DNA fragments, use of DNA Gel
Extraction Kit is recommended.
3. This kit can not remove the template and primers with chain length longer than 50-mer.

Notes

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www.nbsbio.co.uk

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