SpinSmart Plasmid Purification Protocol: High-copy plasmid DNA from E. coli

Denville Scientific

Abstract

SpinSmart Plasmid Miniprep kits are designed to rapidly purify plasmid DNA from bacterial cultures.

The protocol below is appropriate for both CM-410-50 and CM-410-250.

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Guidelines

Spin Smart Plasmid Miniprep Kit Components

Reagent Name	50 preps	250 preps
PB1 Resuspension Buffer	15 ml	75 ml
PB2 Lysis Buffer	15 ml	3 x 25 ml
PB3 Neutralization Buffer	20 ml	100 ml
PB4 Wash Buffer	30 ml	2 x 75 ml
PB5 Wash Buffer EtOH (Concentrate)	2 x 6 ml	2 x 20 ml
PB6 Elution Buffer (5 mM Tris/HCl, pH 8.5)	15 ml	75 ml
Rnase A (Lyophilized)	6 mg	30 mg
SpinSmart Plasmid columns (white ring)	50	250
Collection Tubes (2 ml)	50	250
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Equipment to be supplied by user

96-100% ethanol

1.5 ml microcentrifuge tubes

Manual piEquipment to be supplied by userpettors and disposable pipette tips

Centrifuge for microcentrifuge tubes

Personal protection equipment (lab coat, gloves, goggles)

Product description

SpinSmart Plasmid Miniprep kits are designed to rapidly purify plasmid DNA from bacterial cultures. Bacteria are grown overnight in culture media, then harvested by centrifugation. Pelleted bacterial cells are resuspended in PB1 buffer, then alkaline lysis buffer PB2 releases plasmid DNA from the cells. The reaction is neutralized by PB3 buffer, which also creates appropriate conditions for binding plasmid DNA to the SpinSmart Plasmid Miniprep silica membrane. A centrifugation step pellets precipitated protein, genomic DNA, and cell debris. The supernatant is loaded onto a SpinSmart Plasmid Miniprep binding column.

Salts, metabolites, and soluble cellular debris are removed by wash steps with ethanolic PB5 Wash Buffer. After the wash steps, plasmid DNA is eluted from the column with PB6 Elution Buffer (5 mM Tris/HCl, pH 8.5). If host strains with high levels of nucleases are used, we recommend an additional washing step with pre-warmed PB4 Wash Buffer. The additional wash with PB4 will also increase the reading length of automated fluorescent DNA sequencing reactions.

SpinSmart Plasmid Columns have a DNA binding capacity of 60 µg.

SpinSmart purified plasmid DNA is suitable for applications like automated fluorescent DNA sequencing, PCR, or other enzymatic reactions.

SpinSmart Plasmid Kit Specifications		
Culture volume	1 - 5 ml	high copy
	5 - 10 ml	low copy
Typical yield	< 25 µg	(1 – 5 ml culture)
	< 40 µg	(5 – 10 ml culture)
Elution volume	50 µl	
Binding capacity	60 µg	
Vectors	< 15 kbp	
Time/prep	25 min/18 preps	
Column type	mini spin column	

Growth of bacterial cultures

Plasmid DNA quality and yield is highly dependent on the type of culture media and antibiotics used, the bacterial host strain, the plasmid type, size, and copy number.

LB (Luria Bertani) medium is recommended for standard, high-copy plasmids. The cell culture should be incubated at 37°C with constant shaking (200-250 rpm) preferably 12-16 h overnight. The culture should be grown to an OD600 of 3-6. Rich media, such as 2xYT (Yeast/Tryptone), TB (Terrific Broth) or CircleGrow, can also be used. Bacteria grow faster in rich media, so they reach the stationary phase much earlier than in LB medium (≤ 12 h), and they also achieve higher cell masses. However, this does not necessarily yield more plasmid DNA. Overgrowing a culture could lead to a high percentage of dead or starving cells, which can result in plasmid DNA that is partially degraded or contaminated with chromosomal DNA. Culture medium and incubation time must be optimized for each host strain/plasmid construct combination individually.

Cell cultures must be grown under **antibiotic selection** to ensure plasmid propagation.

Antibiotic Selection Information according to Maniatis*			
Antibiotic	**Stock solution concentration**	**Storage**	**Working concentration**
Ampicillin	50 mg/ml in H_2O	-20°C	50-100 µg/ml
Chloramphenicol	34 mg/ml in EtOH	-20°C	25-170 µg/ml
Kanamycin	10 mg/ml in H_2O	-20°C	10-50 µg/ml
Streptomycin	10 mg/ml in H_2O	-20°C	10-50 µg/ml
Tetracycline	5 mg/ml in EtOH	-20°C	10-50 µg/ml

∗ Maniatis T, Fritsch EF, Sambrook J: Molecular cloning. A laboratory manual, Cold Spring Harbor, Cold Spring, New York 1982.

SpinSmart Plasmid Minipreps recommend to use 5 ml of a well grown bacterial culture, OD_{600} = 3.

Culture volumes can be increased if the cell culture grows very poorly or has to be decreased. Please note the OD_{600} values and corresponding culture values below:

Recommended Culture Volumes

OD_{600}	1	2	3	4	5	6
Culture Volume	15 ml	8 ml	**5 ml**	4 ml	3 ml	2 ml

Elution procedures

High yield: important for larger constructs: Heat elution buffer to 70°C, add 50-100 µl to the SpinSmart Plasmid miniprep column. Incubate at 70°C for 2 min.

High yield: Perform two elution steps with the volume indicated in the individual protocol. 90-100% of bound plasmid DNA can be eluted.

High concentration: Perform one elution step with 60% of the volume indicated in the individual protocol.

High yield and high concentration: Apply half of the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again.

SpinSmart Plasmid Miniprep protocols recommend PB6 Elution Buffer (5 mM Tris, pH 8.5) for elution. We do not recommend buffers containing EDTA, especially if the plasmid DNA is intended for sequencing reactions. Water may be used for elution, however the pH should be adjusted to pH 7.0-8.5 since deionized water typically has a low pH.

Storage and preparation of solutions

Buffers PB3 and PB4 contain guanidine hydrochloride! Wear gloves and goggles when using this kit!

All kit components can be stored at room temperature (20-25°C) and are stable for up to one year.

Keep buffer bottles tightly closed, especially if buffers are warmed at any time.

Sodium dodecyl sulfate (SDS) in PB2 Lysis Buffer may precipitate if stored at temperatures below 20°C. If a precipitate is observed, incubate the bottle at 30–40°C for several minutes and mix well.

Prior to starting the SpinSmart Plasmid Miniprep prepare the following:

Before the first use of the kit, add 1 ml of PB1 Resuspension Buffer to the RNase A vial and vortex briefly. Transfer the mixture into the PB1 bottle and mix thoroughly.
Store PB1 Resuspension Buffer (containing RNase A) at 4°C for up to 6 months.

Add the indicated volume of 96-100% ethanol to PB5 Wash Buffer.

Catalog Number	50 preps Cat. No. CM-410-50	250 preps Cat. No. CM-410-250
Buffer PB5 (Concentrate)	2 x 6 ml add 24 ml 96% - 100% EtOH	2 x 20 ml add 80 ml 96% - 100% EtOH

Safety Information

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
PB2 Lysis Buffer	Sodium hydroxide < 2%	Xi*	Irritating to eyes and skin	R 36/38	S 26-37/39-45
PB3 Neutralization Buffer	Guanidine hydrochloride	Xn**	Harmful if swallowed. Irritating to eyes and skin	R 22-36/38	
PB4 Wash Buffer	Guanidine hydrochloride + isopropanol < 25%	Xn**	Flammable Harmful if swallowed. Irritating to eyes and skin	R 10-22-36/38	S 7-16-25
RNase A	RNase A, lyophilized	Xi**	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24

Risk Phrases

R 10 Flammable

R 22 Harmful if swallowed

R 36/38 Irritating to eyes and skin

R 42/43 May cause sensitization by inhalation and skin contact

Safety Phrases

S 7 Keep container tightly closed

S 16 Keep away from sources of ignition - No Smoking!

S 22 Do not breathe dust

S 24 Avoid contact with the skin

S 25 Avoid contact with the eyes

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice

S 37/39 Wear suitable protective clothing and gloves

S 45 In case of accident or if you feel unwell, seek medical advice immediately

(show the label where possible)

∗ Label not necessary, if quantity below 25 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

∗∗ Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

Troubleshooting

Please see the product manual for troubleshooting information.

https://www.denvillescientific.com/Files/Files/Denville/ProductDocs/CM-410-50_and_M-410-250_manual.pdf

Before start

Prior to starting the SpinSmart Plasmid Miniprep prepare the following:

Before the first use of the kit, add 1 ml of PB1 Resuspension Buffer to the RNase A vial and vortex briefly. Transfer the mixture into the PB1 bottle and mix thoroughly.
Store PB1 Resuspension Buffer (containing RNase A) at 4°C for up to 6 months.

Add the indicated volume of 96-100% ethanol to PB5 Wash Buffer.

Catalog Number	50 preps Cat. No. CM-410-50	250 preps Cat. No. CM-410-250
Buffer PB5 (Concentrate)	2 x 6 ml add 24 ml 96% - 100% EtOH	2 x 20 ml add 80 ml 96% - 100% EtOH

Materials

SpinSmart™ Plasmid Miniprep DNA Purification Kit with Reagents, 50 preps CM-410-50 by Denville Scientific Inc.

Protocol

Cultivate and harvest bacterial cells

Step 1.

Start with 1-5 ml E. coli LB culture*, pellet cells in a microcentrifuge for 30 sec at 11,000 x g

DURATION
00:00:30

NOTES
Nicole Clouse 12 Dec 2016

Culture should be grown to OD_{600} 3-6. **SpinSmart Plasmid Minipreps** recommend to use 5 ml of a well grown bacterial culture, OD_{600} = 3.

Culture volumes can be increased if the cell culture grows very poorly or has to be decreased. Please note the OD_{600} values and corresponding culture values below:

Recommended Culture Volumes

OD_{600}	1	2	3	4	5	6
Culture Volume	15 ml	8 ml	**5 ml**	4 ml	3 ml	2 ml

Cultivate and harvest bacterial cells

Step 2.

Discard the supernatant and remove as much of the liquid as possible.

Cell lysis

Step 3.

Add **250 µl PB1 Resuspension Buffer**.

Cell lysis

Step 4.

Vortex or pipet up and down to resuspend the cell pellet completely. No cell clumps should be visible.

NOTES

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Important: Buffer PB2 should not have SDS precipitate visible prior to use. If a white precipitate is visible, warm the buffer for several minutes at 30-40°C until precipitate is dissolved completely. Cool buffer down to room temperature (20-25°C).

Cell lysis

Step 5.

Add **250 µl PB2 Lysis Buffer.**

Cell lysis

Step 6.

Invert the tube 6-8 times to mix completely. **Do not vortex!**

Cell lysis

Step 7.

Incubate at **room temperature** for up to 5 min or until lysate appears clear.

DURATION

00:05:00

Cell lysis

Step 8.

Add 300 µl PB3 Neutralization Buffer

Cell lysis

Step 9.

Invert the tube 6-8 times to mix completely. **Do not vortex!**

Lysate Clarification

Step 10.

Centrifuge for **5 min** at **11,000 x g** at room temperature.

DURATION

00:05:00

Lysate Clarification

Step 11.

If precipitate is not completely clear from the lysate, centrifuge again for **5 min** at **11,000 x g** at room temperature.

DURATION
00:05:00

Bind DNA
Step 12.

Place a SpinSmart Plasmid Binding Column in a Collection Tube (2 ml) and load a maximum of 750 µl of the supernatant (from the "Lysate Clarification" section above) onto the column.

Bind DNA
Step 13.

Centrifuge for **1 min** at **11,000 x g.**

DURATION
00:01:00

Bind DNA
Step 14.

Discard flow-through and place the SpinSmart Plasmid Binding Column into the Collection Tube (2 ml).

NOTES
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Repeat steps 12-14 of this section to load any remaining lysate.

Wash silica membrane
Step 15.

Optional: For host strains containing high levels of nucleases (e.g. HB101 or strains of the JM series), perform a wash step with **500 µl PB4 Wash Buffer pre-warmed to 50°C.** Centrifuge for **1 min at 11,000 x g** before proceeding with Buffer PB5.

Wash silica membrane
Step 16.

Add 600 µl PB5 Wash Buffer (make sure EtOH has been added).

Wash silica membrane
Step 17.

Centrifuge for 1 min at 11,000 x g.

DURATION

00:01:00

Wash silica membrane

Step 18.

Discard flow-through and place the SpinSmart Plasmid Column back into the **empty** Collection Tube (2 ml).

Dry silica membrane

Step 19.

Centrifuge for 2 min at 11,000 x g and discard the Collection Tube (2 ml).

DURATION

00:02:00

Elute highly pure DNA

Step 20.

Place the SpinSmart Plasmid Binding Column in a 1.5 ml microcentrifuge tube (not provided) and add **50 µl PB6 Elution Buffer.**

NOTES

Nicole Clouse 13 Dec 2016

SpinSmart Plasmid Miniprep protocols recommend PB6 Elution Buffer (5 mM Tris, pH 8.5) for elution. We do not recommend buffers containing EDTA, especially if the plasmid DNA is intended for sequencing reactions. Water may be used for elution, however the pH should be adjusted to pH 7.0-8.5 since deionized water typically has a low pH.

Elute highly pure DNA

Step 21.

Incubate for 1 min at **room temperature.**

DURATION

00:01:00

Elute highly pure DNA

Step 22.

Centrifuge for 1 min at 11,000 x g.

DURATION

00:01:00

Warnings

Buffers PB3 and PB4 contain guanidine hydrochloride! Wear gloves and goggles when using this kit!

Safety Information

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RNase A	RNase A, lyophilized	Xi**	May cause sensitization by inhalation and skin contact	R 42/43	S 22-24

Risk Phrases

R 10 Flammable

R 22 Harmful if swallowed

R 36/38 Irritating to eyes and skin

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Safety Phrases

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