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2 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
User Manual	1	1

Equipment to be supplied by user

96-100% ethanol

1.5 ml microcentrifuge tubes

Manual pipettors and disposable pipette tips

Centrifuge for microcentrifuge tubes

Personal protection equipment (lab coat, gloves, goggles)

3 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

3.1 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 ₂ O	-20°C	50-100 µg/ml
Chloramphenicol	34 mg/ml in EtOH	-20°C	25-170 µg/ml
Kanamycin	10 mg/ml in H ₂ O	-20°C	10-50 µg/ml
Streptomycin	10 mg/ml in H ₂ O	-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

3.2 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.

3.3 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

4 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%	✘ ^o Xi*	Irritating to eyes and skin R 36/38	S 26-37/39-45
PB3 Neutralization Buffer	Guanidine hydrochloride	✘ ^o Xn**	Harmful if swallowed. Irritating to eyes and skin R 22-36/38	
PB4 Wash Buffer	Guanidine hydrochloride + isopropanol < 25%	✘ ^o Xn**	Flammable Harmful if swallowed. Irritating to eyes and skin R 10-22-36/38	S 7-16-25
RNase A	RNase A, lyophilized	✘ ^o 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)

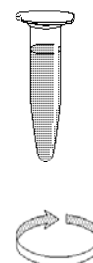
** Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

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

1 Cultivate and harvest bacterial cells

Start with 1-5 ml *E. coli* LB culture*, pellet cells in a microcentrifuge for **30 sec** at **11,000 x g**. Discard the supernatant and remove as much of the liquid as possible.

* Culture should be grown to OD_{600} 3-6 (see page 5)



30 sec
≥ 11,000 x g

2 Cell lysis

Add **250 µl PB1 Resuspension Buffer**. Vortex or pipet up and down to resuspend the cell pellet completely. No cell clumps should be visible.

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).

Add **250 µl PB2 Lysis Buffer**. Invert the tube **6-8 times** to mix completely. **Do not vortex!** Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

Add **300 µl PB3 Neutralization Buffer**. Invert the tube **6-8 times** to mix completely. Do not vortex!



+ 250 µl PB1

resuspend

+ 250 µl PB2

mix

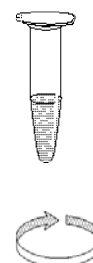
+ 300 µl PB3

mix

3 Lysate Clarification

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

Repeat this step if precipitate is not completely clear from the lysate.



5 - 10 min
≥ 11,000 x g

4 Bind DNA

Place a SpinSmart Plasmid Binding Column in a Collection Tube (2 ml) and load a maximum of 750 µl of the supernatant from step 3 onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the SpinSmart Plasmid Binding Column into the Collection Tube (2 ml).



**load
supernatant**

**1 min
≥ 11,000 x g**

Repeat this step to load any remaining lysate.

5 Wash silica membrane

*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.*

**(Optional)
+ 500 µl PB4**

**1 min
≥ 11,000 x g)**

Add **600 µl PB5 Wash Buffer** (make sure EtOH has been added). Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the SpinSmart Plasmid Column back into the **empty** Collection Tube (2 ml).



+ 600 µl PB5

**1 min
≥ 11,000 x g**

6 Dry silica membrane

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



**2 min
≥ 11,000 x g**

7 Elute highly pure DNA

Place the SpinSmart Plasmid Binding Column in a 1.5 ml microcentrifuge tube (not provided) and add **50 µl PB6 Elution Buffer**. Incubate for **1 min** at **room temperature**. Centrifuge for **1 min** at **11,000 x g**.



**RT
1 min**

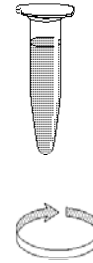
**1 min
≥ 11,000 x g**

6 SpinSmart Plasmid Purification Protocol: Low-copy plasmid DNA, P1 constructs, or cosmids

1 Cultivate and harvest bacterial cells

Start with 5-10 ml *E. coli* LB culture*, pellet cells in a microcentrifuge for **30 sec** at **11,000 x g**. Discard the supernatant and remove as much of the liquid as possible.

* Culture should be grown to OD_{600} 3-6 (see page 5)



30 sec
≥ 11,000 x g

2 Cell lysis

Add **500 µl PB1 Resuspension Buffer**. Vortex or pipet up and down to resuspend the cell pellet completely. No cell clumps should be visible.

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).

Add **500 µl PB2 Lysis Buffer**. Invert the tube **6-8 times** to mix completely. **Do not vortex!** Incubate at **room temperature** for up to **5 min** or until lysate appears clear.

Add **600 µl PB3 Neutralization Buffer**. Invert the tube **6-8 times** to mix completely. Do not vortex!



+ 500 µl PB1

resuspend

+ 500 µl PB2

mix

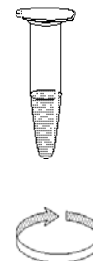
+ 600 µl PB3

mix

3 Lysate Clarification

Centrifuge for **10 min** at **11,000 x g** at room temperature.

Repeat this step if precipitate is not completely clear from the lysate!



10 min
≥ 11,000 x g

4 Bind DNA

Place a SpinSmart Plasmid Binding Column in a Collection Tube (2 ml) and load a maximum of 750 μ l of the supernatant from step 3 onto the column. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the SpinSmart Plasmid Binding Column into the Collection Tube (2 ml).



**load
supernatant**

**1 min
 $\geq 11,000 \times g$**

Repeat this step to load any remaining lysate.

5 Wash silica membrane

Add **500 μ l PB4 Wash Buffer pre-warmed to 50°C**. Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the SpinSmart Plasmid Column back into the **empty** Collection Tube (2 ml).

+ 500 μ l PB4

**1 min
 $\geq 11,000 \times g$**

Add **600 μ l PB5 Wash Buffer** (make sure EtOH has been added). Centrifuge for **1 min** at **11,000 x g**. Discard flow-through and place the SpinSmart Plasmid Column back into the **empty** Collection Tube (2 ml).



+ 600 μ l PB5

**1 min
 $\geq 11,000 \times g$**

6 Dry silica membrane

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



**2 min
 $\geq 11,000 \times g$**

7 Elute highly pure DNA

Place the SpinSmart Plasmid Binding Column in a 1.5 ml microcentrifuge tube (not provided) and add **50 μ l PB6 Elution Buffer pre-warmed to 70°C**. Incubate for **2 min** at **70°C**. Centrifuge for **1 min** at **11,000 x g**.



**70°C
2 min**

**1 min
 $\geq 11,000 \times g$**

7 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of bacterial cells	Cell pellet not properly resuspended
	<i>The cell pellet must be completely resuspended prior to lysis. No cell clumps should be visible before addition of PB2 Lysis Buffer.</i>
	<i>SDS in PB2 Lysis Buffer may precipitate upon storage. If a visible precipitate is formed, incubate Buffer PB2 at 30–40°C for 5 min and mix well.</i>
	Too many bacterial cells used
	<i>LB is recommended as optimal growth medium. When using rich media like Terrific Broth, cultures may overgrow quickly and the cell density may be too high.</i>
Poor plasmid yield	Incomplete Lysis of bacterial cells (see above)
	Suboptimal precipitation of SDS and cell debris
	<i>Centrifuging at 4°C instead of room temperature may improve precipitation of SDS and cell debris.</i>
	<i>Insufficient amounts of antibiotic used during cultivation</i>
	<i>Cells carrying the plasmid of interest may become overgrown by non-transformed cells when inadequate levels of the appropriate antibiotic are used. Add appropriate amounts of freshly prepared antibiotic solutions to all media.</i>
	Bacterial culture too old
	<i>Do not incubate cultures for more than 16 h at 37°C under shaking. When using rich media like Terrific Broth, cultivation time should be reduced to < 12 h.</i>

Problem

Possible cause and suggestions

Poor plasmid yield (continued)	<p>Suboptimal elution conditions</p> <p><i>Use a slightly alkaline elution buffer like PB6 Elution Buffer (5 mM Tris/HCl, pH 8.5). Elution efficiencies drop drastically with buffers below pH 7.0, or if using water that is acidic. Any buffer or water used for elution should be between pH 7.0-8.5.</i></p> <p><i>Low copy-number plasmids (e.g. plasmids bearing the P15A ori, cosmids, or P1 constructs) require larger culture volumes, we recommend between 5 – 10 ml.</i></p>
No plasmid yield	<p>Reagents not applied properly</p> <p><i>Add indicated volume of 96-100% ethanol to PB5 Wash Buffer.</i></p> <p>Nuclease-rich host strains are used</p> <p><i>If using nuclease-rich strains like E. coli HB101 or strains of the JM series, be sure to perform the optional PB4 Wash Buffer step. Optimal endonuclease removal can be achieved by incubating the membrane with pre-warmed Buffer PB4 Wash Buffer (50°C) for 2 min before centrifugation.</i></p> <p><i>When working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation.</i></p> <p>Inappropriate storage of plasmid DNA</p> <p><i>Store plasmid DNA dissolved in water at < -18°C or at < +5°C when dissolved in PB6 Elution Buffer or TE buffer.</i></p>

Problem

Possible cause and suggestions

Nicked plasmid DNA

Cell suspension was incubated with alkaline PB2 Lysis Buffer for more than 5 min.

Genomic DNA contamination

Poor plasmid quality

Cell lysate was vortexed or mixed too vigorously after addition of PB2 Lysis Buffer. Genomic DNA was sheared and was then able to bind to the membrane with plasmid DNA.

Smearred plasmid bands on agarose gel

Store plasmid preparations on ice or frozen in order to avoid DNA degradation..

Carryover of ethanol

Make sure to centrifuge ≥ 1 min at 11,000 x g in step 6 for complete removal of ethanolic PB5 Wash Buffer.

Elution of plasmid DNA with TE buffer

EDTA may inhibit sequencing reactions. Elute with PB6 Elution Buffer or water, pH 7.0 – 8.5.

Suboptimal performance in enzymatic reactions

No additional washing with Buffer PB4 performed

Additional washing with 500 μ l PB4 Wash Buffer before washing with ethanolic PB5 Wash Buffer will increase the reading length of sequencing reactions.

Not enough DNA used for sequencing reaction

Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.

Plasmid DNA prepared from too much bacterial cell material

*Do not use more than 3 ml of a saturated *E. coli* culture if preparing plasmid DNA for automated fluorescent DNA sequencing.*

8 Ordering information

Cat. No.	Description	Qty.	Price
CM-410-50	SpinSmart™ Plasmid Miniprep DNA Purification Kit	50 preps	\$79.00
CM-410-250	SpinSmart™ Plasmid Miniprep DNA Purification Kit	250 preps	349.00
CM-400-50	Plasmid Miniprep Bulk Columns with Collection Tubes only	50 per pack	49.00
CM-400-250	Plasmid Miniprep Bulk Columns with Collection Tubes only	250 per pack	219.00