

SpinSmart HS Maxi Plasmid Purification Kit

Instruction Manual

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Caution: Always wear gloves and follow proper lab safety procedures when handling chemicals and reagents.

I. Introduction

The SpinSmart HS Maxi plasmid purification kit is designed for purification of up to 1.2 mg of high quality plasmid DNA from a starting volume of 200 - 250 ml of bacterial culture. The principle of the kit is based on the modified alkaline lysis technique for plasmid DNA purification. The kit provides a simple and fast procedure with environment-friendly buffers. High quality plasmid DNA produced by the kit are suitable for many applications such as restriction digestion, PCR, in vitro transcription, probe synthesis and DNA sequencing.

Here are a few important benefits of using the SpinSmart HS Maxi plasmid purification kit:

- **No high speed centrifugation is needed.** Other kits require high speed centrifugation for clearing of lysate precipitation, and for alcohol precipitation of plasmid DNA.
- **The procedure is simple and can be completed in about 50 min.** If a vacuum manifold is used, the entire purification process can be completed in about **35 min.** Other kits' protocols take more than two hours to complete.
- **Environment-friendly buffer without chaotropic salt or guanidine.** Other kits use chaotropic salt or guanidine in their purification buffer.
- **Column wash is carried out by 70% ethanol.** Other kits use wash buffers containing salts which can inhibit downstream enzymatic applications.
- **Purified plasmid DNA is directly eluted into TE buffer or water at the final step.** Unlike other plasmid purification kits, the SpinSmart HS Maxi plasmid purification kit does not require alcohol precipitation and DNA pellet resuspension steps, which often lead to a salt problem that could inhibit downstream enzymatic applications.

II. Component List

- 1) Buffer A (130 ml)
Dissolve RNase A with 1 ml of Buffer A by pipetting. Add the content to Buffer A and mix well before using the buffer. **Store the solution at 4 °C after the addition of RNase A.**
- 2) Buffer B (130 ml)
Store the solution at room temperature. **Check the solution and make sure it is clear. Precipitation will form in the bottle below 18 °C. Significant reduction in yield can occur if all the components in the solution are not dissolved completely.** If the solution is cloudy, warm the solution at 37 °C and mix the content until the solution is clear before use.
- 3) Buffer C2 (130 ml)
Store the solution at room temperature. **Check the solution and make sure it is clear.** If the solution is not clear due to precipitation, warm the solution at 37 °C and mix the content until the solution is clear before use.
- 4) RNase A (one vial)
Dissolve RNase A with 1 ml of Buffer A by pipetting. Add the content to Buffer A and mix well before using the buffer. **Store the solution at 4 °C after the addition of RNase A.**
- 5) DNA binding column unit (10 units)
- 6) Lysate filtering syringe (10 syringes)
- 7) TE buffer (30 ml)
Store at room temperature.
- 8) 2 ml centrifuge tubes (10 tubes) for plasmid DNA storage.
- 9) 50 ml centrifuge tubes (10 tubes) for plasmid DNA elution.
- 10) Instruction manual.

III. Additional Materials and Equipment Required

- 1) Low speed centrifuge with swing-bucket rotor (preferred) or fixed-angle rotor that can hold standard 50 ml centrifuge tubes, 250 ml or 500 ml centrifuge bottles (See section VI for conversion of centrifugal force (in units of g) to RPM (revolution in minute) for your rotor).
- 2) Optional: Vacuum manifold for plasmid purification.
- 3) 250 ml or 500 ml centrifuge bottles for pelleting bacteria from culture.
- 4) New, sterile 50 ml centrifuge tubes.
- 5) Heat block or water bath for heating TE or water to 65 - 70 °C.
- 6) 95% or 100% ethanol (molecular biology grade).
- 7) Spectrophotometer for DNA quantitation.

IV. Plasmid DNA Purification Procedures

A. Standard Procedures

Note: Unless it is specified, the steps described below are performed at room temperature.

1. Inoculate 200 - 250 ml LB containing appropriate antibiotic in a 1-liter flask with 0.1 ml of an overnight culture of *E. Coli* containing the desired plasmid. Grow the culture at 37 °C for 12 - 16 hours with vigorous shaking (200 - 300 rpm).
2. Transfer the culture to a 250 ml or 500 ml centrifuge bottle. Pellet down the bacteria by centrifugation for 10 min. at 3,500 x g.
3. Resuspend the bacterial pellet in **10 ml** of Buffer A by pipetting (Complete resuspension of bacteria is critical for high yield). **Be sure to add RNase A into Buffer A before use.**
4. Transfer the suspension to a 50 ml centrifuge tube. Add **10 ml** of Buffer B (**make sure no precipitaion in the solution**). Close the cap and mix the tube gently and thoroughly by inverting the tube 20 times. Let it stand at room temperature for 5 min. The mixture should become clear and viscous. **Do Not Vortex! Vortexing shears genomic DNA and leads to genomic DNA contamination.**
5. Add **2 ml** of Buffer C2 to the tube. Close the cap and gently mix the solution by inverting the tube 20 times. White precipitation should appear. **Do Not Vortex! Vortexing shears genomic DNA and leads to genomic DNA contamination.**
6. Centrifuge the mixture at 3,500 x g for 5 min.
7. Place a new 50 ml centrifuge tube on a rack. Remove the syringe plunger, position the filtering syringe on top of the centrifuge tube. Pass the supernatant from step 6 (it is OK to have some floating precipitation in the supernatant) through the filtering syringe and into the 50 ml centrifuge tube.
8. Add **8 ml** of Buffer C2 and **6.5 ml** of 95 % or 100% ethanol to the filtrate in the 50 ml centrifuge tube. Close the cap and mix gently and thoroughly by inverting the tube 20 times.
9. Transfer **15 ml** of the mixture from step 8 to the DNA binding column. Close the cap and centrifuge the unit for 3 min at 3,500 x g. Carefully

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remove the binding column from the unit and discard the liquid from the collection tube. Reassemble the DNA binding unit.

10. Repeat step 9 for the remaining mixture from step 8.
11. Add **15 ml** of **70%** ethanol to the DNA binding column. Close the cap and centrifuge the unit for 3 min. at 3,500 x g. Carefully remove the binding column from the binding unit and discard the filtrate from the collection tube. Reassemble the DNA binding unit.
12. Repeat step 11 (total two washes).
13. Add **15 ml** of **95% or 100%** ethanol to the DNA binding column. Centrifuge the unit for 3 min at 3,500 x g. Carefully remove the binding column from the binding unit and discard the filtrate from the collection tube. Reassemble the DNA binding unit.
14. Centrifuge the unit for an additional 10 min. at 3,500 x g.
Optional step for automated DNA sequencing: Centrifuge the unit for 20 min instead of 10 min. at 3,500 x g. Open the cap of the unit and let it stand for 10 min. to ensure complete removal of ethanol. Small amount of leftover ethanol from the wash buffer can inhibit automated DNA sequencing
15. Transfer the DNA binding column to a new 50 ml centrifuge tube (included in the kit). Add **1 ml** of preheated (65 - 70 °C) TE or sterile water (not included) to the center of the DNA binding column and let it stand at room temperature for 1 min. **For DNA sequencing or any other salt sensitive applications such as difficult ligation or transformation, elute the plasmid DNA with sterile water instead of TE. For long term storage, plasmid DNA suspended in water needs to be stored frozen (-20 °C) to prevent hydrolyzation.**
16. Close the cap, elute the plasmid DNA from the binding column by centrifuging the unit for 5 min. at 3,500 x g. **Optional step: For maximal recovery of plasmid DNA, repeat steps 15 - 16. 65 - 75% of plasmid DNA is released from the column in the first elution. If high concentration of plasmid DNA is desired do not combine the first elution with the second elution. Use the first elution for applications requiring high concentration of plasmid DNA.**
17. Transfer the eluted plasmid DNA from the collecting tube to a 2 ml tube (included in the kit) and store the DNA at 4 °C if it is eluted with TE, or - 20 °C if it is eluted with water.

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B. Vacuum Manifold Produces

Note: Unless it is specified, the steps described below are performed at room temperature.

1. Inoculate 200 - 250 ml LB containing appropriate antibiotic in a 1-liter flask with 0.1 ml of an overnight culture of *E. Coli* containing the desired plasmid. Grow the culture at 37 °C for 12 - 16 hours with vigorous shaking (200 - 300 rpm).
2. Transfer the culture to a 250 ml or 500 ml centrifuge bottle. Pellet down the bacteria by centrifugation for 10 min. at 3,500 x g.
3. Resuspend the bacterial pellet in **10 ml** of Buffer A by pipetting (Complete resuspension of bacteria is critical for high yield). **Be sure to add RNase A into Buffer A before use.**
4. Transfer the suspension to a 50 ml centrifuge tube. Add **10 ml** of Buffer B (**make sure no precipitaion in the solution**). Close the cap and mix the tube gently and thoroughly by inverting the tube 20 times. Let it stand at room temperature for 5 min. The mixture should become clear and viscous. **Do Not Vortex! Vortexing shears genomic DNA and leads to genomic DNA contamination.**
5. Add **2 ml** of Buffer C2 to the tube. Close the cap and gently mix the solution by inverting the tube 20 times. White precipitation should appear. **Do Not Vortex! Vortexing shears genomic DNA and leads to genomic DNA contamination.**
6. Centrifuge the mixture at 3,500 x g for 5 min.
7. Place a new 50 ml centrifuge tube on a rack. Remove the syringe plunger, position the filtering syringe on top of the centrifuge tube. Pass the supernatant from step 6 (it is OK to have some floating precipitation in the supernatant) through the filtering syringe and into the 50 ml centrifuge tube.
8. Add **8 ml** of Buffer C2 and **6.5 ml** of 95 % or 100% ethanol to the filtrate in the 50 ml centrifuge tube. Close the cap and mix gently and thoroughly by inverting the tube 20 times.
9. Remove the DNA binding column from the unit. Connect it to a vacuum manifold and turn on the vacuum.
10. Pass the mixture from step 8 through the column. After the mixture

has passed through the column completely, leave the vacuum on for one more min.

11. Pass **30 ml** of **70%** ethanol through the column. After the ethanol has passed through the column completely, leave the vacuum on for one additional min.
12. Pass **15 ml** of **95 or 100%** ethanol through the column. After the ethanol has passed through the column completely, leave the vacuum on for one additional min.
13. Reassemble the DNA binding unit.
14. Centrifuge the unit for an additional 10 min. at 3,500 x g.
Optional step for automated DNA sequencing: Centrifuge the unit for 20 min instead of 10 min. at 3,500 x g. Open the cap of the unit and let it stand for 10 min. to ensure complete removal of ethanol. Small amount of leftover ethanol from the wash buffer can inhibit automated DNA sequencing
15. Transfer the DNA binding column to a new 50 ml centrifuge tube (included in the kit). Add **1 ml** of preheated (65 - 70 °C) TE or sterile water (not included) to the center of the DNA binding column and let it stand at room temperature for 1 min. **For DNA sequencing or any other salt sensitive applications such as difficult ligation or transformation, elute the plasmid DNA with sterile water instead of TE. For long term storage, plasmid DNA suspended in water needs to be stored frozen (-20 °C) to prevent hydrolyzation.**
16. Close the cap, elute the plasmid DNA from the binding column by centrifuging the unit for 5 min. at 3,500 x g. **Optional step: For maximal recovery of plasmid DNA, repeat steps 15 - 16. 65 - 75% of plasmid DNA is released from the column in the first elution. If high concentration of plasmid DNA is desired do not combine the first elution with the second elution. Use the first elution for applications requiring high concentration of plasmid DNA.**
17. Transfer the eluted plasmid DNA from the collecting tube to a 2 ml tube (included in the kit) and store the DNA at 4 °C if it is eluted with TE, or - 20 °C if it is eluted with water.

V. Troubleshooting

Q. Low yield

- A. 1) Too many cells and not enough buffer to lyse cells completely. After resuspension of cell pellet in Buffer A (step 3), the final total volume should not exceed 12 ml. If the volume exceeds 12 ml, double the volume of Buffer A, B, C2 and ethanol recommended in the purification procedures to ensure complete lysis.
- 2) Working with low copy plasmid. Harvest twice the amount of cell culture. Growing cells in rich medium such as *Terrific Broth* can increase the density of cells. Remember to use twice the volume of Buffer A, B, C2 and ethanol recommended in the purification procedures to ensure complete lysis.
- 3) Make sure the cells are resuspended in Buffer A (step 3) completely. Incomplete resuspension of cells decreases the efficiency of lysis.
- 4) Make sure there is no precipitation in Buffer B and C2. Precipitation in these buffers decreases the efficiency of lysis. Warm the solutions at 37 °C. Vortex or shake well to redissolve the precipitants if necessary.

Q. Contamination of high molecular weight chromosomal DNA

- A. During steps 4 - 5, samples should not be vortexed or shaken vigorously. Also step 4 (lysis step) should not exceed more than 10 min. Both can cause shearing of the genomic DNA and lead to high molecular weight chromosomal DNA contamination.

Q. Sample contains RNA

- A. 1) Too many cells were harvested. Use twice the amount of Buffer A, B, C2 and ethanol in the purification process.
- 2) RNase A activity is weakened. Buffer A with RNase A should be stored at 4°C to maintain its full activity. Full RNase A activity can be obtained within 6 months after the addition of RNase A in Buffer A and the buffer is properly stored at 4°C. Add additional RNase A (200 µg/ml) to Buffer A if the RNase A activity is reduced or lost.

Q. Sample floats upon loading in agarose gel

- A. Sample contains ethanol from the washing step. Make sure to follow step 14 closely. If problem persists, spin the unit for 20 min. instead of 10 min. as recommended. Open the cap of the unit and

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let the column air dry for 30 min. at room temperature before elution.

- Q. O.D. ratio between 260 nm and 280 nm above 2.1
- A. The kit normally produces plasmid DNA with O.D. ratio between 1.8 to 2.1. If the ratio is above 2.1,
- 1) Washing (steps 11 - 14) was not completed. Repeat steps 11 - 14 one more time.
 - 2) Sample contains ethanol from the washing step. Make sure step 14 is followed closely. If problem persists, spin the unit for 20 min. instead of 10 min. as recommended. Open the cap of the unit, and air dry the column for 30 min. at room temperature before elution.
- Q. Purified plasmid DNA is degraded.
- A. 1) *E. coli* strains like HB101, BL21(DE3), JM101, JM110, LE392, stb13, TG1 are endA⁺ strains and carry high level of endonuclease activity that cannot be completely removed by the purification process and can lead to DNA degradation. Use endA⁻ *E. coli* strains such as DH5 α , XL-1 blue, TOP10, JM107, JM109, sure2 as host.

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VI. Conversion Of Centrifugal Force (In Units Of g) To RPM (Revolution In Minute)

Formula:

$$\text{Centrifugal Force (g)} = (1.118 \times 10^{-5}) \times (\text{rotor radius in cm}) \times \text{RPM}^2$$

Common Rotors

Rotor	Rotor radius(cm)	Centrifuge bottle/tube size	3,500 x g	
Beckman S4180	16.1	180 ml	4,400 rpm	
	TS-5.1-500	19.0	500 ml	4,100 rpm
	TA-10-250	13.7	250 ml	4,800 rpm
	JA-10	15.8	500 ml	4,500 rpm
	JA-14	13.7	250 ml	4,800 rpm
	JA-20	10.8	50 ml	5,400 rpm
	JS-7.5	16.5	250 ml	4,400 rpm
	JS-13	14.0	50 ml	4,700 rpm
	Sorvall	HB-4	14.7	50 ml
HB-6		14.6	50 ml	4,600 rpm
HS-4		17.2	250 ml	4,300 rpm
GSA		14.5	250 ml	4,700 rpm
GS3		15.1	500 ml	4,600 rpm
SA-300		9.7	50 ml	5,700 rpm
SA-600		12.9	50 ml	4,900 rpm
SL-50T		10.7	50 ml	5,400 rpm
SLA -1500		13.6	250 ml	4,800 rpm
SS-34	10.7	50 ml	5,400 rpm	

VII. Appendix

LB broth

Mix 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl in 500 ml of high quality water. Adjust the pH of the medium to 7.5 with NaOH. Add water to the medium to a final volume of 1000 ml. Sterilize the medium by autoclaving for 20 min.