GENETIX BRAND

HANDBOOK

Nucleo-pore®

Purification of Plasmid DNA

Large Plasmid (<15 kb)
Low copy Plasmid and Cosmids

☐	**SureSpin® Plasmid Mini Kit**	NP-37105	50 Preps
☐	**SureSpin® Plasmid Mini Kit**	NP-37107	250 Preps
☐	**SureSpin® Plasmid FastPrep Kit**	NP-47105	50 Preps
☐	**SureSpin® Plasmid FastPrep Kit**	NP-47107	250 Preps

V 2.12

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Nucleo-pore®

Contents

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SureSpin® Plasmid Mini kit Catalogue no.	50 Preps NP-37105	250 Preps NP-37107
SureSpin® Columns (White Ring)	50	250
Collection Tubes (2 ml)	50	250
Resuspension Buffer PA1	15 ml	75 ml
Lysis Buffer PA2	15 ml	3 x 25 ml
Neutralization Buffer PA3	20 ml	100 ml
Wash Buffer PW	30 ml	2 x 75 ml
Wash Buffer PW4 (Concentrate)	2 x 6 ml	2 x 20 ml
Elution Buffer PAE*	15 ml	75 ml
Rnase A (Lypholized)	6mg	30mg
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SureSpin® Plasmid FastPrep kit Catalogue no.	50 Preps NP-47105	250 Preps NP-47107
SureSpin® FastPrep Columns (Orange Ring)	50	250
Collection Tubes (2 ml)	50	250
Resuspension Buffer PA1	15 ml	75 ml
Lysis Buffer PA2	15 ml	3 x 25 ml
Neutralization Buffer PA3	20 ml	100 ml
Wash Buffer PQ (Concentrate)	6 ml	2 x 20 ml
Elution Buffer PAE*	15 ml	75 ml
Rnase A (Lypholized)	6mg	30mg
Handbook	1	1

**Composition of Elution Buffer PPAE: 5 mM Tris/HCl, pH 8.5*

STORAGE

SureSpin® Plasmid Mini and SureSpin® Plasmid FastPrep Kits should be stored dry at room temperature (15–25°C). Kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage these kits can be kept at 2–8°C. If any precipitate forms in the buffers after storage at 2–8°C it should be redissolved by warming the buffers to 37°C before use. After addition of RNase A, Buffer PA1 is stable for 6 months when stored at 2–8°C. RNase A stock solution can be stored for two years at room temperature.

INTRODUCTION

Principle and Procedures

The SureSpin® Plasmid kit or SureSpin® Plasmid FastPrep kit or is based on alkaline lysis of Bacterial cells followed by adsorption of DNA onto silica in the presence of high salt. The unique silica membrane used in SureSpin® column or SureSpin® FastPrep Columns completely replaces glass or silica slurries for plasmid minipreps.

The procedure consists of three basic steps:

- Preparation and clearing of a bacterial lysate
- Adsorption of DNA onto the SureSpin® column membrane
- Washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

Preparation and clearing of a bacterial lysate

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis to liberate the plasmid DNA. The resulting lysate is neutralized to create appropriate conditions for binding of plasmid DNA on the silica membrane in the SureSpin® column or SureSpin® FastPrep Column.

Adsorption of DNA onto the SureSpin® column or SureSpin® FastPrep Column membrane.

Cell debris and SDS precipitate are pelleted by centrifugation, and the supernatant containing the plasmid DNA is loaded onto the SureSpin® column or SureSpin® FastPrep Column which use a silica membrane for selective adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis procedure, combined with the unique silica membrane, ensure that only DNA will be adsorbed, while RNA, cellular proteins, and metabolites are not retained on the membrane but are found in the flow-through.

Washing and elution of plasmid DNA

The adsorbed DNA is washed to remove contaminants like salt, metabolites and soluble macromolecular cellular components and finally DNA is eluted under low ionic strength conditions with slightly alkaline Elution Buffer (5 mM Tris-HCl, pH 8.5). The purified Plasmid DNA is suitable for applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

DNA yield

Plasmid yield with the SureSpin® system varies depending on plasmid copy number per cell, the individual insert in a plasmid, factors that affect growth of the bacterial culture, the elution volume, and the elution incubation time. To obtain the optimum combination of DNA quality, yield, and concentration, we recommend using Luria-Bertani (LB) medium for growth of cultures, eluting plasmid DNA in a volume of 50 µl, and performing a short incubation after addition of the elution buffer.

Specifications of SureSpin® Plasmid mini / FastPrep Kit

The SureSpin® Plasmid mini and SureSpin® Plasmid FastPrep kits are designed for the rapid, small-scale preparation of highly pure plasmid DNA (mini preps).

The SureSpin® Plasmid Column offer a very high DNA binding capacity of up to 60 µg. This, however, requires thorough washing. Therefore, the kit includes an additional Wash Buffer PW which is strongly recommended for host strains with high levels of endonucleases like ABLE, HB101, or JM110.

The SureSpin® FastPrep Column features a new specially treated silica membrane which allows speeding up the procedure by a combined washing and drying step. No additional steps are necessary if nuclease rich host strains are used. The number of washing and drying steps is reduced from 3 to only 1, therefore the hands-on-time is less than 11 min. However, the DNA binding capacity is limited to 15 µg.

IMPORTANT NOTES

Please read the following notes before starting any of the SureSpin® procedures.

Growth of bacterial cultures in tubes or flasks

- **Pick a single colony from a freshly streaked selective plate and inoculate a culture of 1–5 ml LB medium containing the appropriate selective antibiotic. Incubate for 12–16 h at 37°C with vigorous shaking.**
  Growth for more than 16 h is not recommended since cells begin to lyse and plasmid yields may be reduced. Use a tube or flask with a volume of at least 4 times the volume of the culture.
- **Harvest the bacterial cells by centrifugation at 11,000 rpm in a conventional, table-top microcentrifuge for 3 min at room temperature (15–25°C).**

Buffer notes

- Add the provided RNase A solution to Buffer PA1, mix, and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PW4 and Buffer PQ before use (see bottle label for volume).
- Check Buffers PA2 and PA3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer PA2 vigorously.
- Close the bottle containing Buffer PA2 immediately after use to avoid acidification of Buffer PA2 from CO2 in the air.
- Buffers PA3, and PW contain irritants. Wear gloves when handling these buffers.

Centrifugation notes

- All centrifugation steps are carried out at 11,000 rpm in a conventional, table-top microcentrifuge.

Elution Notes

- Ensure that the elution buffer is dispensed directly onto the center of the SureSpin® membrane for optimal elution of DNA.
- For increased DNA yield, use a higher elution-buffer volume. For increased DNA concentration, use a lower elution-buffer volume.
- For high yield of large constructs, heat elution buffer to 70°C, add 50 -100 µl to the SureSpin® Plasmid column and incubate at 70°C for two minutes.
- Elution Buffer PAE (5 mM Tris/HCl, pH 8.5) can be replaced by TE buffer or water as well. However, we recommend using a weakly buffered, slightly alkaline buffer containing no EDTA, especially if the plasmid DNA is intended for sequencing reactions. If water is used, the pH should be checked and adjusted to pH 8.0 – 8.5 since deionized water usually exhibits a pH below 7.

SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs).

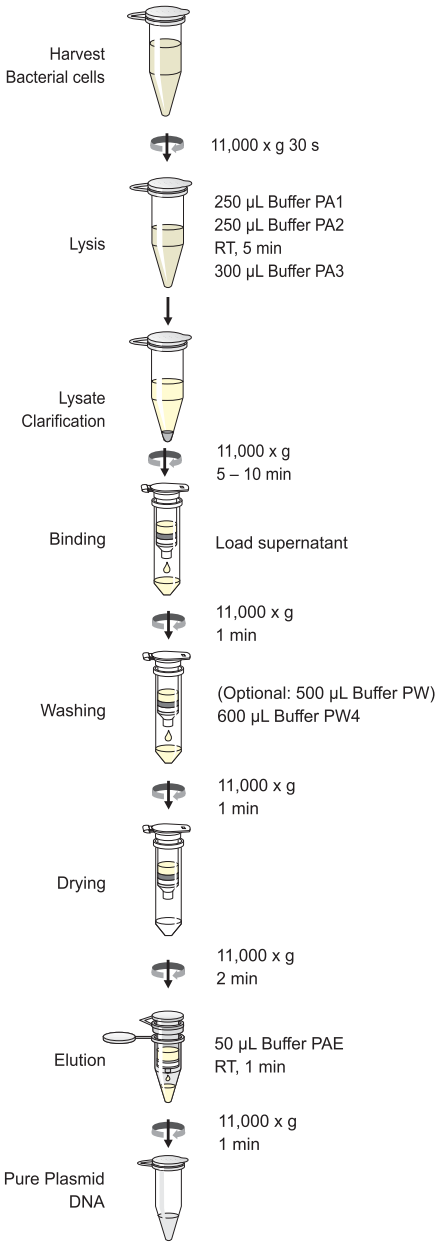
CAUTION: Do not add bleach or acidic solutions directly to the sample-preparation waste. Buffers PA3 and PW contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to **SureSpin® Plasmid mini** or **SureSpin® FastPrep Kit**

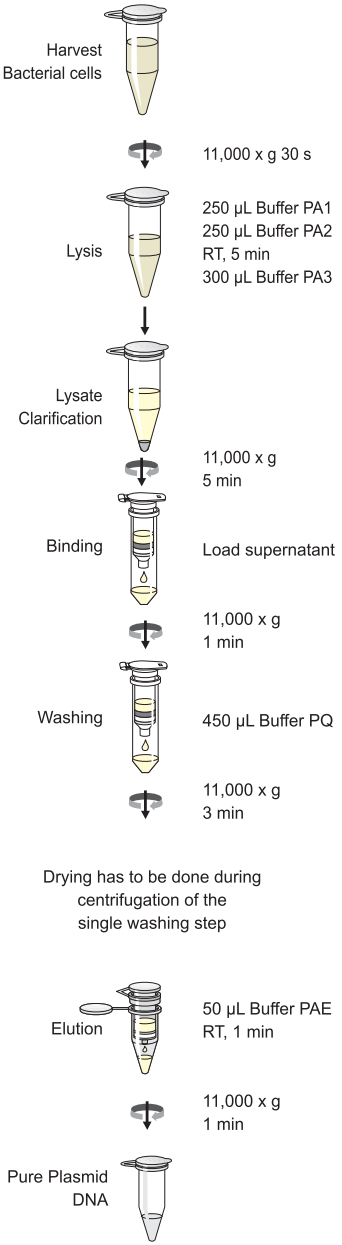
Buffer PA3
Contains guanidine hydrochloride, acetic acid: harmful, irritant. Risk and safety phrases:* R22-36/38, S 7-16-25.
Buffer PA2
Contains sodium hydroxide < 2% : irritant. Risk and safety phrases:* R36/38, S-26-37/39-45.
Buffer PW
Contains guanidine hydrochloride, isopropanol: harmful, flammable, irritant. Risk and safety phrases:* R10-22-36/38.
RNase A
Contains ribonuclease: sensitizer. Risk and safety phrases:* R42/43, S22-24.

R 10 Flammable R 36/38 Irritating to eyes and skin R 22 Harmful if swallowed R 42/43 May cause sensitization by inhalation and skin contact 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)

SureSpin® Plasmid Mini Kit



SureSpin® Plasmid FastPrep kit



Purification of high-copy plasmid DNA from E. coli using SureSpin® Plasmid Mini Kit

This protocol is designed for purification of up to 25 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium.

Please read "Important Notes" on Pg no. 5 before starting.
Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Use 1– 5 ml of a saturated E. coli LB culture, pellet cells for 30 s at 11,000 x g in table-top microcentrifuge..**
   Discard the supernatant and remove as much of the liquid as possible.

2. **Resuspend pelleted bacterial cells in 250 µl Buffer PA1 and transfer to a microcentrifuge tube.**
   Ensure that RNase A has been added to Buffer PA1. No cell clumps should be visible after resuspension of the pellet.

3. **Add 250 µl Buffer PA2 and mix thoroughly by inverting the tube 5–7 times.**
   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

4. **Add 300 µl Buffer PA3 and mix immediately and thoroughly by inverting the tube 5–7 times.**
   To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer PA3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.

5. **Centrifuge for 5 min at 11,000 rpm in a table-top microcentrifuge.**
   A compact white pellet and clear supernatant will form. Repeat this step if supernatant in not clear.

6. **Apply the supernatants from step 5 to the SureSpin® Plasmid Column by decanting or pipetting.**
   Place a SureSpin® Plasmid Column in a Collection Tube (2 ml) and decant the supernatant from step 5 or pipette a maximum of 750 µl of the supernatant onto the column. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the SureSpin® Plasmid Column back into the collection tube. Repeat this step to load the remaining lysate.

7. **Wash the SureSpin® column by adding 500 µl Buffer PW and centrifuging for 30–60 s. Discard the flow-through.**

   This step is necessary to remove trace nuclease activity when using end A+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5a do not require this additional wash step.

   It is strongly recommended performing an additional washing step with 500 µl Buffer PW preheated to 50 °C and centrifuge for 1 min at 11,000 x g before proceeding with Buffer PW4. Additional washing with Buffer PW will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions.

   Add 600 µl Buffer PW4 (supplemented with ethanol), Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the SureSpin® Plasmid Column back into the empty collection tube.

8 **For drying of silica membrane centrifuge for 2 min. at 11000 x g and discard the collection tube.**

   Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.

9 **To elute DNA, place the SureSpin® column in a clean 1.5 ml microcentrifuge tube and add 50 µl Buffer PAE (5 mM Tris·Cl, pH 8.5) or water to the center of each SureSpin® column, let stand for 1 min, and centrifuge for 1 min.**

   Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) see Elution notes pg. no 5

Purification of low-copy plasmids, P1 constructs, or cosmids using SureSpin® Plasmid Mini Kit

This protocol is designed for purification of low-copy plasmid DNA from 5–10 ml overnight cultures of *E. coli* in LB(Luria-Bertani) medium. Processing of larger culture volumes requires increased lysis buffer volumes. The buffer volumes provided with the kit are calculated for high-copy plasmid purification only. Thus, if this support protocol is to be used frequently, an additional SureSpin® Buffer Set can be ordered separately.

Please read "Important Notes" on Pg no. 5 before starting.
Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Use 5– 10 ml of a saturated E. coli LB culture, pellet cells for 30 s at 11,000 x g in table-top microcentrifuge.**
   Discard the supernatant and remove as much of the liquid as possible.

2. **Resuspend pelleted bacterial cells in 500 µl Buffer PA1 and transfer to a microcentrifuge tube.**
   Ensure that RNase A has been added to Buffer PA1. No cell clumps should be visible after resuspension of the pellet.

3. **Add 500 µl Buffer PA2 and mix thoroughly by inverting the tube 5–7 times.**
   Note: Check Buffer PA2 for precipitated SDS 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 (18 – 25 °C). Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

4. **Add 600 µl Buffer PA3 and mix immediately and thoroughly by inverting the tube 5–7 times.**
   To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer PA3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.

5. **Centrifuge for 10 min at 11,000 rpm in a table-top microcentrifuge.**
   A compact white pellet and clear supernatant will form. Repeat this step if supernatant in not clear.

6. **Apply the supernatants from step 5 to the SureSpin® Plasmid Column by decanting or pipetting.**
   Place a SureSpin® Plasmid Column in a Collection Tube (2 ml) and decant the supernatant from step 5 or pipette a maximum of 750 µl of the supernatant onto the column. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the SureSpin® Plasmid Column back into the collection tube. Repeat this step to load the remaining lysate.

7. **Wash the SureSpin® column by adding 500 µl Buffer PW and centrifuging for 30–60 s. Discard the flow-through.**
   This step is necessary to remove trace nuclease activity when using end A+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5a do not require this additional wash step.
   It is strongly recommended performing an additional washing step with 500 µl Buffer PW preheated to 50 °C and centrifuge for 1 min at 11,000 x g before proceeding with Buffer PW4. Additional washing with Buffer PW will also increase the reading length of DNA sequencing reactions and improve the performance of critical enzymatic reactions. Add 600 µl Buffer PW4 (supplemented with ethanol), Centrifuge for 1 min at 11,000 x g.
   Discard flow-through and place the SureSpin® Plasmid Column back into the empty collection tube.

8. **For drying of silica membrane, centrifuge for 2 min at 11,000 x g and discard the collection tube.**
   Note: Residual ethanolic wash buffer might inhibit enzymatic reactions.

9. **To elute DNA, place the SureSpin® Plasmid Column in a 1.5 ml microcentrifuge tube (not provided) and add 50 µl Buffer PAE preheated to 70 °C Incubate for 2 min at 70°C. Centrifuge for 1 min at 11,000 x g.**
   Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water) please see Elution notes at pg 5

Purification of high-copy plasmid DNA from E. coli SureSpin® Plasmid FastPrep Kit

This protocol is designed for purification of up to 15 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of E. coli in LB (Luria-Bertani) medium.

Please read "Important Notes" on Pg no. 5 before starting.
Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Use 1 – 5 ml of a saturated E. coli LB culture, pellet cells for 30 s at 11,000 x g in table-top microcentrifuge .**
   Discard the supernatant and remove as much of the liquid as possible.

2. **Resuspend pelleted bacterial cells in 250 µl Buffer PA1 and transfer to a microcentrifuge tube.**
   Attention: *Check Buffer PA2 for precipitated SDS 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 (18 – 25 °C).*
   Ensure that RNase A has been added to Buffer PA1. No cell clumps should be visible after resuspension of the pellet.

3. **Add 250 µl Buffer PA2 and mix thoroughly by inverting the tube 5–7 times.**
   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

4. **Add 300 µl Buffer PA3 and mix immediately and thoroughly by inverting the tube 5–7 times.**
   To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer PA3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.

5. **Centrifuge for 5 min at 11,000 rpm in a table-top microcentrifuge.**
   A compact white pellet and clear supernatant will form. Repeat this step if supernatant in not clear.

6. **Apply the supernatants from step 5 to the SureSpin® Plasmid Column by decanting or pipetting.**
   Place a SureSpin® Plasmid Column in a Collection Tube (2 ml) and decant the supernatant from step 5 or pipette a maximum of 750 µl of the supernatant onto the column. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the SureSpin® Plasmid Column back into the collection tube. Repeat this step to load the remaining lysate.

7. **Add 450 µl Buffer PQ (supplemented with ethanol, see important notes pg. #).**
   Centrifuge for 3 min at 11,000 x g. Very carefully discard the collection tube and the flowthrough and make sure the spin cup outlet does not touch the wash buffer surface. Otherwise repeat the centrifugation step.

8. **Drying of the SureSpin® Plasmid Fast Column is performed by the 3 min centrifugation in step 7.**

9. **To elute DNA, place the SureSpin® Plasmid Column in a 1.5 ml microcentrifuge tube (not provided) and add 50 µl Buffer PAE.**
   Incubate for 1 min at room temperature. Centrifuge for 1 min at 11,000 x g.
   Note: For more efficient elution procedures and alternative elution buffer (e.g., TE buffer or water), please see Elution Notes at Pg. No 5

Purification of plasmids from Gram-positive bacteria from SureSpin® Plasmid and SureSpin® Plasmid FastPrep Kit

It is necessary to start the lysis procedure with an enzymatic treatment (e.g., Lysozyme, Lysostaphin, Mutanolysin) to break up the peptidoglycan layers for plasmid purification from bacteria with a more resistant cell wall (e.g., Bacillus, Staphylococcus). For some Gram-positive bacteria (e.g., Bifidobacteria, Corynebacteria) even a Pre-incubation with lysozyme might be insufficient and mechanical cell disruption methods have to be used (e.g., RiboLyser).

1. **Use up to 5 ml (SureSpin® Plasmid) or 3 ml (SureSpin® Plasmid FastPrep) of a saturated** *E. coli* **LB culture, pellet cells for 30 s at 11,000 x g in a table-top microcentrifuge.** Discard the supernatant and remove as much liquid as possible.

2. Add **250 µl Buffer PA1** containing **10 mg / ml Lysozyme** (not provided with the kit). Resuspend the cell pellet completely by vortexing or pipetting up and down. Make sure no cell clumps remain in the suspension.
   Incubate at 37 °C for 10 – 30 minutes.
   Proceed with addition of Buffer PA2 in step 2 of the protocol for isolation of high-copy plasmids from *E. coli* with SureSpin® Plasmid or SureSpin® Plasmid FastPrep.

Plasmid DNA clean-up using SureSpin® Plasmid and SureSpin® Plasmid FastPrep Kit

Plasmid or DNA fragment preparations from other origins than bacterial cells, for example, enzymatic reactions, can be purified using SureSpin® Plasmid or SureSpin® Plasmid FastPrep by omitting the cell lysis step.

Before starting the preparation:
Check if Wash Buffer PW4 or Buffer PQ were prepared according to important notes at Pg # .

1. **Add 2 volumes of Buffer PA3 to 1 volume of DNA solution and mix well by vortexing.** (e.g., add 200 µl Buffer PA3 to 100 µl enzymatic reaction mix.) to adjust binding conditions.

2 **Place a SureSpin® Plasmid or Plasmid FastPrep Column in a Collection Tube (2 ml) and load the mixture onto the column.**
   Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the SureSpin® Plasmid Column or Plasmid FastPrep Column back into the collection tube.
   Note: Maximum loading capacity of the SureSpin® Plasmid Column or Plasmid FastPrep Column is 750 µl. Repeat the procedure if larger volumes are to be processed.

   Proceed with the washing steps of the protocol for isolation of high-copy plasmids from *E. coli* with SureSpin® Plasmid or SureSpin® Plasmid FastPrep Kit.

SUPPLEMENTARY PROTOCOL
Purification of Plasmid DNA from Yeast

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure.

Additional reagents needed:

- Wash Buffer: 10 mM EDTA, pH 8.0
- Sorbitol Buffer (prepare fresh): 1.2 M sorbitol, 10 mM $CaCl_2$, 0.1 M Tris/HCl pH 7.5, 35 mM β-mercaptoethanol
- Lyticase or zymolase

1. **Harvest yeast cells**
   Centrifuge **3 mL YPD yeast culture** (OD_{600} up to 10) for 10 min at 5,000 x g and discard supernatant.

2. **Wash cells**
   Resuspend cells in **1 mL Wash Buffer**. Centrifuge for **10 min** at **5,000 x g** and discard supernatant.

3. **Resuspend pellet**
   Resuspend cells in **600 µL Sorbitol Buffer**.

4. **Digest cell wall**
   Add **200 U lyticase** and incubate for **30 min at 30 °C** (spheroplast formation).

5. **Isolate spheroplasts**
   Centrifuge for **10 min at 2,000 x g** and discard supernatant.

6. **Isolate plasmids**
   Continue with step 2 of the SureSpin® Plasmid standard protocol with addition of **250 µL Buffer PA2**.

TROUBLESHOOTING GUIDE

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in Genetix Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see back cover for contact information).

Bacterial cells lysis is incomplete

Possible cause

- Cell resuspension incomplete

Suggestion(s)

- It is important that the cell pellet is completely resuspended prior to lysis. Cell clumps should not be visible before addition of Buffer PA2.

Possible cause

- Buffer PA2 precipitated

Suggestion(s)

- Upon storage, SDS in Buffer PA2 may precipitate. If a precipitate is formed, incubate Buffer PA2 at 30 – 40 °C for 5 min and mix well.

Possible cause

- Too many bacterial cells used

Suggestion(s)

- LB is recommended as optimal growth medium. When using very rich media like TB (terrific broth), the cell density of the cultures may become too high.

Low Yield

Possible cause

- Incomplete bacterial cells lysis

Suggestion(s)

- See suggestions as explained above.

Possible cause

- Antibiotic amount insufficient

Suggestion(s)

- Cells carrying the plasmid of interest may become overgrown by non-transformed cells, when insufficient of the appropriate antibiotics are used. Add freshly prepared stock solutions and appropriate amount of antibiotics to both solid and liquid media.

Low Yield

Possible cause

- Old Bacterial culture

Suggestion(s)

- Do not incubate cultures for more than 16 h at 37°C under shaking condition. LB is recommended as optimal growth medium; however, if using very rich media like TB (terrific broth), cultivation time should be reduced to < 12h.

Possible cause

- Elution conditions

Suggestion(s)

- Use a slightly alkaline elution buffer like Buffer PAE (5 M Tris / HCl, pH 8.5). When using nuclease free water for elution, make sure that pH value is within range of 7-8.5.

Possible cause

- Low copy-number plasmid was used

Suggestion(s)

- For low copy number plasmids (e.g., plasmids with P15A ori, cosmids, or P1 constructs), the culture volumes should be increased to at least 5 ml.

Low DNA quality

Possible cause

- Nicked plasmid DNA

Suggestion(s)

- Incubation of cell suspension with alkaline Lysis Buffer PA2 for more than 5 min.

Possible cause

- Genomic DNA contamination

Suggestion(s)

- Lysate was vortexed or mixed too vigorously after addition of Buffer PA2. Genomic DNA was sheared and thus liberated.

Possible cause

- Smeared DNA bands

Suggestion(s)

- When working with nuclease-rich strains, do plasmid preparations on ice or frozen in order to avoid DNA degradation. Be sure to perform the optional PW washing step If using nuclease rich strains. Optimal endonuclease removal can be achieved by incubating the membrane with preheated Buffer PW (50 °C) for 2 min before centrifugation.

Suboptimal performance of plasmid DNA in downstream applications

Possible cause

- Eluate contains residual ethanol

Suggestion(s)

- Make sure to centrifuge ≥1 min at 11,000 x g in step 7 in order to achieve complete removal of ethanolic Buffer PW4 for SureSpin® Plasmid Mini kit and centrifuge ≥3 min at 11,000 x g in step 8 in order to achieve complete removal of ethanolic Buffer PQ for SureSpin® Plasmid FastPrep Kit.

Possible cause

- Elution of plasmid DNA with TE buffer

Suggestion(s)

- EDTA may inhibit sequencing reactions. Repurify plasmid DNA and elute with Buffer PAE or water. Alternatively, the eluted plasmid DNA can be precipitated with ethanol and redissolved in Buffer PAE or water.

Possible cause

- No Additional washing

Suggestion(s)

- Additional washing with 500 µl Buffer PW before washing with ethanolic Buffer PW4 will increase the reading length of sequencing reactions for SureSpin® Plasmid Mini kit.

Possible cause

- DNA quantity not enough for sequencing reaction

Suggestion(s)

- Quantify DNA before setting up sequencing reactions.

Possible cause

- Plasmid DNA prepared from too much bacterial cell material

Suggestion(s)

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

APPENDIX

Growth of bacterial cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic. The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium.

Plasmid copy number

Plasmids vary widely in their copy number per cell, depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell.

Origins of replication and copy numbers of various plasmids

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1*	500–700	high copy
pBluescript vectors	ColE1	300–500	high copy
pGEM® vectors	pMB1*	300–400	high copy
pTZ vectors	pMB1*	>1000	high copy
pBR322 and derivatives	pMB1*	15–20	low copy
pACYC and derivatives	p15A	10–12	low copy
pSC101 and derivatives	pSC101	~5	very low copy
Cosmids			
SuperCos	ColE1	10–20	low copy
pWE15	ColE1	10–20	low copy

The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy-number plasmids listed here contain mutated versions of this origin.

Host strains

Most E. coli strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid has an effect on the quality of the purified DNA. Host strains such as DH1, DH5α, and C600 give high-quality DNA. The slower growing strain XL1-Blue also yields DNA of very high-quality which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM series, produce large amounts of carbohydrates, which are released during lysis and can inhibit enzyme activities if not completely removed. In addition, these strains have high levels of endonuclease activity which can reduce DNA quality. The methylation and growth characteristics of the strain should also be taken into account when selecting a host strain. XL1-Blue and DH5α are highly recommended for reproducible and reliable results.

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures may lead to uneven plasmid yield or loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid.

The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent so that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibioticresistant clone can be picked. A single colony should be inoculated into 1–5 ml of media containing the appropriate selective agent, and grown with vigorous shaking for 12–16 hours. Growth for more than 16 hours is not recommended since cells begin to lyse and plasmid yields may be reduced.

Antibiotics

Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the par locus which ensures that the plasmids segregate equally during cell division. Daughter cells that do not receive plasmids will replicate much faster than plasmid-containing cells in the absence of selective pressure, and can quickly take over the culture.

The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the plasmid linked bla gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. Ampicillin is also very sensitive to temperature, and when in solution should be stored frozen in single-use aliquots. The recommendations given in below Table are based on these considerations.

Concentrations of commonly used antibiotics

Antibiotic	Stock solutions		Working concentration (dilution)
	Concentration	Storage	
Ampicillin (sodium salt)	50 mg/ml in water	–20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	–20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in water	–20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	–20°C	50 µg/ml (1/100)

Culture media

Luria-Bertani (LB) broth is the recommended culture medium for use with SureSpin® plasmid Kits, since richer broths such as TB (Terrific Broth) or 2x YT lead to extremely high celldensities, which can overload the purification system. It should be noted that cultures grown in TB may yield 2–5 times the number of cells compared to cultures grown in LB broth. If these media are used, recommended culture volumes must be reduced to match the capacity of the SureSpin® membrane. If excess culture volume is used, alkaline lysis will be inefficient, the SureSpin® membrane will be overloaded, and the performance of the system will be unsatisfactory. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, which may result in shearing of bacterial genomic DNA and contamination of the plasmid DNA. Care must also be taken if strains are used which grow unusually fast or to very high cell densities. In such cases, doubling the volumes of Buffers is recommended. It is best to calculate culture cell density and adjust the volume accordingly.

Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are in common use. Although different LB broths produce similar cell densities after overnight culture, plasmid yields can vary significantly.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep® Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

Product Warranty

SureSpin® Plasmid Mini Kit and SureSpin® Plasmid Fast Prep Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the SureSpin® Plasmid Mini Kit and SureSpin® Plasmid Fast Prep Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

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