GENETIX BRAND

HANDBOOK

Nucleo-pore®

Purification of Ultrapure, Transfection Grade Plasmid DNA

☐	**SurePrep® Plasmid Mini Kit**	NP-15123	20 Preps
☐	**SurePrep® Plasmid Mini Kit**	NP-15125	100 Preps
☐	**SurePrep® Plasmid Midi Kit**	NP-15143	20 Preps
☐	**SurePrep® Plasmid Midi Kit**	NP-15145	100 Preps
☐	**SurePrep® Plasmid Maxi Kit**	NP-15161	10 Preps
☐	**SurePrep® Plasmid Maxi Kit**	NP-15162	25 Preps
☐	**SurePrep® Plasmid Mega Kit**	NP-15183	5 Preps
☐	**SurePrep® Plasmid Giga Kit**	NP-15191	5 Preps

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Nucleo-pore®

Contents

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KIT CONTENTS

SurePrep®Plasmid Kit Pack Size	Mini 20 preps	Mini 100 preps	Midi 20 preps	Midi 100 preps	Maxi 10 preps	Maxi 25 preps	Mega 5 preps	Giga 5 preps
Catalog no.	**NP-15123**	**NP-15125**	**NP-15143**	**NP-15145**	**NP-15162**	**NP-15163**	**NP-15181**	**NP-15191**
Resuspension Buffer PRB1	25 ml	2 x 25 ml	100 ml	2 x 250 ml	150 ml	2 x 200 ml	250 ml	750 ml
Lysis Buffer PLB2	25 ml	2 x 25 ml	4 x 25 ml	2 x 250 ml	150 ml	400 ml	250 ml	750 ml
Neutralization Buffer PNB3	25 ml	2 x 25 ml	100 ml	2 x 250 ml	150 ml	400 ml	250 ml	750 ml
Equilibration Buffer PQB2	25 ml	125 ml	70 ml	2 x 150 ml	70 ml	200 ml	140 ml	250 ml
Wash Buffer PWB3	3 x 30 ml	3 x 125 ml	250 ml	3 x 400 ml	2 x 250 ml	2 x 500 ml	2 x 250 ml	1000ml
								2x200 ml
Elution Buffer PEB5	32 ml	120 ml	120 ml	3 x 200 ml	200 ml	500 ml	200 ml	500 ml
								120 ml
RNase A (lyophilized)*	2.5 mg	2 x 2.5 mg	10 mg	2 x 25 mg	15 mg	2 x 25 mg	25 mg	80 mg
SurePrep® Mini Columns	20	100	-	-	-	-	-	-
SurePrep® Midi Columns	-	-	20	100	-	-	-	-
SurePrep® Maxi Columns	-	-	-	-	10	25	-	-
SurePrep® Mega Columns	-	-	-	-	-	-	5	-
SurePrep® Giga columns	-	-	-	-	-	-	-	5
SurePrep® Folded Filters	-	-	20	100	10	25	5	10 type1
								10 Type2
Plastic Washers	10	10	10	10	5	10	5	-
Hand Book	1	1	1	1	1	1	1	1

STORAGE

SurePrep® Plasmid 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 PRB1 is stable for 6 months when stored at 2–8°C. RNase A stock solution can be stored for two years at room temperature.

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).

The following risk and safety phrases apply to components of the SurePrep® Plasmid Purification Plasmid Kits.

Buffer PLB2

Contains sodium hydroxide: irritant. Risk and safety phrases:* R36/38, S 26-37/39-45

RNase A, lypholized

Contains ribonuclease: sensitizer. Risk and safety phrases: *R42/43, S 22-24

*R 36/38 Irritating to eyes and skin; R 42/43 May cause sensitization by inhalation and skin contact; S 22 Do not breathe dust; S 24 Avoid contact with the skin; S 26 In case of contact with eyes, rinse immediately with plenty of water and seek; S 37/39 Wear suitable gloves and eye / face protection; S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

INTRODUCTION

Principle and Procedure

SurePrep® Plasmid Purification Kits are based on the remarkable selectivity of silica based Anion-Exchange Resin, allowing purification of ultrapure supercoiled plasmid DNA with high yields. Anion-exchange–based SurePrep® Plasmid kits yield transfection grade DNA, which is highly suited for use in a broad variety of demanding applications such as transfection, *in vitro* transcription, translation, and all enzymatic modifications.

SurePrep® Plasmid Purification Kits protocols are based on a modified alkaline lysis procedure, followed by binding of plasmid DNA to SurePrep® Anion-Exchange Resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Plasmid DNA is eluted in a high-salt buffer and then concentrated and desalted by isopropanol precipitation. Each disposable column is packed with SurePrep® Resin is designed to operate by gravity flow, reducing the amount of hands-on time required for the purification procedure.

Specifications of SurePrep® Plasmid Purification Kits

SurePrep® Plasmid Purification Kits contain SurePrep® columns of different sizes Mini, Midi, Maxi, Mega and Giga, appropriate buffers and RNase A. SurePrep® columns are Polypropylene columns containing SurePrep® Anion-Exchange silica resin packed between two inert filter elements which can be used over a wide range of pH 2.5-8.5 and can remain intact with buffers for upto three hours without any change in its chromatographic properties. The resin remains functional in buffers containing upto 2M salt and remains intact in the presence of denaturing agents like formamide, urea and common reagents like Triton X-100 and NP-40. SurePrep® Columns are resistant to organic solvents such as alcohol, chloroform and phenol and free from DNase and RNase.

Equipments and Reagents to be supplied by user

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- Centrifugation tubes or vessels with suitable capacity for the volumes specified in the appropriate protocol.
- Refrigerated centrifuge capable of ≥20,000 x g with rotor for the appropriate centrifuge tubes or bottles
- SurePrep® rack or equivalent holder
- Ice
- Isopropanol
- 70% ethanol
- Buffer to reconstitute plasmid DNA (e.g., TE buffer, pH 8.0, or sterile water)

Important Notes

It is highly recommended to read the handbook carefully before beginning the DNA Preparation. Experienced users may refer to the Flow chart. The Flow chart is designed to be used as supplemented tool for quick reference during the purification procedure.

Plasmid Size

The protocols are suitable for purifying most plasmids ranging from 3 – 10 kbp, cosmids from 10 – 50 kbp and very large constructs (P1 constructs, BACs, PACs) up to 300 kbp.

Plasmid Copy number

SurePrep® Plasmid kits are recommended for the isolation of high-copy plasmids (> 20 copies per cell), however, low-copy plasmids (< 20 copies per cell) can be isolated as well. If you are purifying low-copy plasmids you will need to supplement the SurePrep® Plasmid kits with additional buffers. We recommend the SurePrep® Buffer Set I for routine purification of low-copy plasmids.

Culture Media

The cultivation of bacterial cells is recommended at 37 °C in LB medium at constant shaking (200 – 250 rpm). Rich media like 2 x YT (Yeast / Tryptone) or TB (Terrific Broth) can also be used. By using 2 x YT or TB, bacteria grow faster and reach the stationary phase much earlier than in LB medium (≤ 12 h). This may lead to a higher percentage of dead or starving cells when starting the preparation. The resulting plasmid DNA from overgrown cultures may be partially degraded or contaminated with chromosomal DNA. For Mini and Midi preps cultivation in flasks is recommended. For Mega preps the use of an appropriate fermentation system is recommended in order to optimize cultivation conditions.

Culture volume

Yield and quality of plasmid DNA depend on the type of growing media and antibiotics, the bacterial host, plasmid type, size, or copy number. Therefore, these factors should be taken into consideration. For cultivation of bacterial cells, we recommend LB medium. The suggested bacterial culture volumes for each column size as well as expected plasmid yields are listed in Table 2. Overnight cultures in flasks usually reach, under vigorous shaking, an OD600 of 3 – 6, while fermentation cultures reach 10 and more. Therefore, please refer not only to the culture volume, but also check OD600 and pellet wet weight, particularly if richer culture media like 2 x YT or TB are used. If too much bacterial material is used, lysis and precipitation steps are inefficient and finally SurePrep® Columns are overloaded causing decreased yield and plasmid quality.

Note: 1 liter E. coli culture grown in LB medium yields a pellet of about 3 – 20 g wet weight. The expected yield for a high-copy-number plasmid is 1 – 3 mg per gram wet weight.

High copy plasmids

Culture Volume	wet pellet weight	SurePrep® Columns	yield
1-5 ml	-	Mini	3-20 µg
5-30 ml	-	Midi	20-100 µg
30-1500 ml	0.75g	Maxi	100-500 µg
150-500 ml	2.5 g	Mega	500 µg- 2 mg
500-2,000 ml	10 g	Giga	2 mg-10 mg

Low copy plasmids

Culture volume	wet pellet weight	SurePrep® Columns	yield
3-10 ml	-	Mini	3-20 µg
10-100 ml	-	Midi	20-100 µg
100-500 ml	1.5-2.2 g	Maxi	100-500 µg
500-2,000 ml	5-7.5 g	Mega	500 µg-2 mg

Note: For Mini and Midi, depending on the media used , OD600 should be determined.

Binding capacity of SurePrep® Columns

SurePrep® Columns are available in a variety of sizes for preparation of as little as 20 µg or as much as 10 mg plasmid DNA. The maximum plasmid binding capacities of the SurePrep® Columns Mini, Midi, Maxi, Mega, and Giga are at least 20µg, 100µg, 500µg, 2 mg, and 10 mg respectively. Actual yields will depend on culture volume, culture medium, plasmid copy number, size of insert, and host strain.

Convenient stopping points in protocols

For all protocols, the purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets can be stored at –20°C for several weeks. In addition, the DNA eluted from the SurePrep® Columns can be stored overnight at 2–8°C, after which the protocol can be continued.

Lysate filtration

Once the alkaline lysis is over, the solution has to be clarified from e.g. the cell debris through the supplied **SurePrep® Folded Filters** or **SurePrep® Bottle Top Filters** in order to prevent clogging of the **SurePrep® columns**.

SurePrep® Folded Filters are provided for filtration of the lysate. Folded filters are designed to eliminate the centrifugation step after alkaline lysis for plasmid isolation. The filters completely remove SDS and cellular debris from plasmid samples. Furthermore folded filters avoid shearing of large DNA constructs, such as PACs or BACs. Folded Filters should not be used with Anion-Exchange Mini columns due to the small culture volumes which allow an easy and quick clearing of the lysate in a microcentrifuge. To handle large culture volumes two SurePrep® Folded Filters (Type 1 and Type 2) are included in the **SurePrep® plasmid Giga** kit in order to guarantee an optimal removal of SDS and cellular debris from plasmid sample.

For the Anion- Exchange Mega and Giga columns the vacuum operated bottle top filters can also be used for filtration of the lysate. The **SurePrep® Bottle Top Filters** make the separation of the bacterial lysate and SDS precipitate easily, quickly, and conveniently. When using a SurePrep® Bottle Top Filter it is not necessary to centrifuge the solution first, as described in step 7, option 1and 2 of the corresponding protocol. Adjust the bottle top filter to a suitable flask (e.g., Schott), load the bacterial lysate and apply the vacuum. After 3 – 5 min the solution will have passed through. Load the resulting clear lysate onto the corresponding **SurePrep® Anion-Exchange Column** and discard the bottle top filter.

Buffer Notes

Before you start any SurePrep® plasmid DNA purification prepare the following:

- Dissolve the lyophilized RNase A by the addition of 1 ml Resuspension Buffer PRB1.
- Wearing gloves is recommended. Pipette up and down until the RNase A is dissolved completely. Transfer the RNase A solution back to the bottle containing Buffer PRB1 and shake well. Indicate date of RNase A addition. The final concentration of RNase A is 100 µg / ml Buffer PRB1.
- Store Buffer PRB1 with RNase A at 4 °C. The solution will be stable at this temperature for at least 6 months.
- Lysis Buffer PLB2 should be stored at room-temperature (18 – 25 °C) since the containing SDS may precipitate at temperatures below 20 °C. If precipitation occurs, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is redissolved.

Elution Notes

DNA should be Eluted in a new tube with the volume of elution buffer indicated in the corresponding protocol. The plasmid DNA is precipitated with the addition of room temperature (18 – 25 °C) isopropanol. Do not let the plasmid DNA solution drop into a vial with isopropanol, because this leads to spontaneous co-precipitation of salt.

Use room-temperature (18 – 25 °C) isopropanol to prevent spontaneous coprecipitation of salt.

SurePrep® Plasmid Kits

	MINI	MIDI	MAXI	MEGA	GIGA
Harvest Bacterial Cells	4,500 – 6,000 x g 15 min at 4°C	4,500 – 6,000 x g 15 min at 4°C	4,500 – 6,000 x g 15 min at 4°C	4,500 – 6,000 x g 15 min at 4°C	4,500 – 6,000 x g 15 min at 4°C
	High copy / low-copy				
Lysis – Buffer PRB1	0.4 ml / 0.8 ml	4 ml / 8 ml	12 ml / 24 ml	45 ml / 90 ml	120 ml / -
Lysis – Buffer PLB2	0.4 ml / 0.8 ml RT, < 5 min	4 ml / 8 ml RT, < 5 min	12 ml / 24 ml RT, < 5 min	45 ml / 90 ml RT, < 5 min	120 ml / - RT, < 5 min
Lysis – Buffer PNB3	0.4 ml / 0.8 ml 0 °C, 5 min	4 ml / 8 ml 0 °C, 5 min	12 ml / 24 ml 0 °C, 5 min	45 ml / 90 ml 0°C, 5 min	120 ml / - 0 °C, 5 min
Column Equilibration	Buffer PQB2 1 ml	Buffer PQB2 2.5 ml	Buffer PQB2 6 ml	Buffer PQB2 20 ml	Buffer PQB2 100 ml
Lysate clarification	Centrifugation 12,000 x g 15 min	Folded Filter or centrifugation 12,000 x g 25 min	Folded Filter or centrifugation 12,000 x g 40 min	Folded Filter or centrifugation 12,000 x g 50 min	Folded Filter or centrifugation 12,000 x g 60 min
Binding	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column
Washing	Buffer PWB3 High copy 2 x 1.5 ml Low copy 2 x 2 ml	Buffer PWB3 High copy 10 ml Low copy 12 ml.	Buffer PWB3 High copy 32 ml Low copy 2 x 18 ml	Buffer PWB3 High copy 2 x 35 ml Low copy 2 x 50 ml	Buffer PWB3 High copy 2 x 100 ml
Elution	Buffer PEB5 1 ml	Buffer PEB5 5 ml	Buffer PEB5 15 ml	Buffer PEB5 25 ml	Buffer PEB5 100 ml
Precipitation	Isopropanol 0.75 ml	Isopropanol 3.5 ml	Isopropanol 11 ml	Isopropanol 18 ml	Isopropanol 70 ml
	≥ 15,000 x g 30 min at 4 °C	≥ 15,000 x g 30 min at 4 °C	≥ 15,000 x g 30 min at 4 °C	≥ 15,000 x g 30 min at 4 °C	≥ 15,000 x g 30 min at 4 °C
DNA Pellet Washing & Drying	70 % ethanol 500 µl	70 % ethanol 2 ml	70 % ethanol 5ml	70 % ethanol 7 ml	70 % ethanol 10 ml
	≥15,000 x g 10 min at RT 5 – 10 min	≥15,000 x g 10 min at RT 5 – 10 min	≥15,000 x g 10 min at RT 10 – 20 min	≥15,000 x g 10 min at RT 30 – 60 min	≥15,000 x g 10 min at RT 30 – 60 min
Reconstitute Plasmid DNA	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE	Appropriate volume of TE

Purification of high-copy plasmid DNA using SurePrep® Plasmid Mini, Midi, Maxi Kit

- Please read Important Notes at Pg no. 5-7 before starting.
- Black (Marked with a ■) denotes values for SurePrep® Mini kit; Blue (marked with a ▲) denotes values for SurePrep® Plasmid Midi and Red (marked with a ●) denotes values for SurePrep® Plasmid Maxi kit.

Procedure

1. **Pick a single colony from freshly streaked selective plate and inoculate the appropriate volume of LB medium containing the selective antibiotic. Incubate for approx. 12-16 hrs at 37°C with vigorous shaking.**
   Use a tube or flask with a volume of at least 4 times the volume of the culture

2. **Harvest the bacterial cells by centrifugation at 4,500 – 6000 x g for 15 min at 4 °C.**
   If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

3. **Resuspend the bacterial pellet in ■0.4 ml ▲4 ml ●12 ml Buffer PRB1.**
   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer PRB1.

4. **Add ■0.4 ml ▲4 ml ●12 ml Buffer PLB2 to the suspension. Mix gently by inverting the tube 6 - 8 times.**
   Incubate the mixture at room temperature (18 – 25 °C) for 2 – 3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

5. **Add ■0.4 ml ▲4 ml ●12 ml pre-cooled Buffer PNB3 (4 °C) to the suspension.**
   Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. Incubate the suspension on ice for 5 min.

6. **Equilibrate a SurePrep® Mini, Midi or Maxi Column with 1.0 ml 2.5 ml 6.0 ml Buffer PQB2.**
   Allow the column to empty by gravity flow. Discard the flow through.

7. **Clear the bacterial lysate by following either option 1 (Midi, Maxi) or option 2 (Mini, Midi, Maxi), described below.**
   This step is extremely important; excess precipitate left in suspension may clog the SurePrep® Column in later steps.
   Option 1. Filter the suspension. Place a SurePrep® Folded Filter in a small funnel for support, and prewet the filter with a few drops of Buffer PQB2 or sterile deionized water. Load the bacterial lysate onto the wet filter and collect the flowthrough.
   Important: Do not use SurePrep® Folded Filters with Mini columns (Mini preps).

   Option 2. Centrifuge the suspension. Centrifuge at > 12,000 x g for the minimum time ■ 15 min ▲ 25 min ● 40 min at 4 °C. If the suspension contains residual precipitate after the first centrifugation, either repeat this step or proceed with option 1.

8. **Load the cleared lysate from step 7 onto the SurePrep® Column.**
   Allow the column to empty by gravity flow.

9. **Wash the column with ■ 2 x 1.5 ml ▲ 10 ml ● 32 ml Buffer PWB3.**
   Repeat as indicated. Discard the flow-through.

10. **Elute the plasmid DNA with ■ 1 ml ▲ 5 ml ● 15 ml Buffer PEB5.**
    We recommend precipitating the eluate as soon as possible (step 11). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.
    Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 13).

11. **Add ■ 0.75 ml ▲ 3.5 ml ● 11.0 ml isopropanol to precipitate the eluted plasmid DNA.**
    Mix carefully and centrifuge at ≥ **15,000 x g for 30 min at 4°C.** Carefully discard the supernatant.

12. **Add ■ 500 µl ▲ 2 ml ● 5 ml 70 % ethanol to the pellet.**
    Vortex briefly and centrifuge at ≥ **15,000 x g for 10 min at room temperature (18 – 25°C)**. Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry at **room temperature (18 – 25 °C) for ■ 5 – 10 min ▲ 5 – 10 min ● 10 – 20 min** not less than the indicated time.
    Drying for longer periods of time will not harm the quality of plasmid DNA but overdrying may render the DNA less soluble.

13. **Dissolve pellet in an appropriate volume of buffer TE or sterile deionized water to reconstitute the DNA.**
    Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).

    Note: Determine plasmid yield by UV Spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

Purification of high-copy plasmid DNA using SurePrep® Plasmid Mega and Giga Kit

- Please read Important Notes at Pg no. 5-7 before starting.
- Blue (marked with a ▲) denotes value for SurePrep® Plasmid Mega and Red (marked with a ●) denotes value for SurePrep® Plasmid Giga kit.

Procedure

1. **Pick a single colony from freshly streaked selective plate and inoculate the appropriate volume of LB medium containing the selective antibiotic. Incubate for approx. 12-16 hrs at 37°C with vigorous shaking.**
   Use a tube or flask with a volume of at least 4 times the volume of the culture

2. **Harvest bacteria from an LB culture by centrifugation at 4,500 – 6,000 x g for 15 min at 4°C.**

3. **Carefully resuspend the pellet of bacterial cells in ▲ 45 ml ● 120 ml Buffer PRB1 + RNase A.**

4. **Add ▲ 45 ml ● 120 ml Buffer PLB2 to the suspension.**
   Mix gently by inverting the tube 6 – 8 times. Incubate the mixture at room temperature (18 – 25 °C) for 2 – 3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

5. **Add pre-cooled ▲ 45 ml ● 120 ml Buffer PNB3 (4 °C) to the suspension.**
   Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. Incubate the suspension on ice for 5 min.

6. **Equilibrate a SurePrep® Mega, Giga Column with ▲ 20 ml ● 100 ml Buffer PQB2**.
   Allow the column to empty by gravity flow. Discard flow-through.

7. Clear the bacterial lysate by following either **option 1 or option 2**, described below. This step is extremely important; excess precipitate left in suspension may clog the SurePrep® Column in later steps.

   Option 1. Place a SurePrep® Folded Filter in a large funnel for support, and prewet the filter with a few drops of Buffer PQB2 or sterile deionized H_2O. Load the bacterial lysate onto the wet filter and collect the flow -through.

   Note: SurePrep® Giga kits contain two types of folded filters (Type 1 and Type 2) in order to guarantee an optimal and fast filtration. Put folded filter Type 1 into folded filter Type 2 and prewet the filters with a few drops of Buffer PQB2 or sterile deionized H2O before loading lysate.

   For Giga columns, we recommend dividing the samples in half to clear the lysate: use two SurePrep® Folded Filters and two funnels simultaneously. Then combine the flow-through before proceeding with step 8 .

   For Mega and Giga columns alternatively the vacuum operated SurePrep® Bottle Top Filters (not included) can be used for filtration of the lysate.

   Option 2. Centrifuge at > 12,000 x g for the **minimum 50 min 60 min** at 4°C. If the suspension contains residual precipitate after the first centrifugation, either repeat this step or proceed with option 1.

8. **Load the cleared lysate from step 7 onto the SurePrep® Column. Allow the column to empty by gravity flow.**

   Optional: You may want to save all or part of the flow-through for analysis.

9. **Wash the column with ▲2 x 35 ml ●2 x 100 ml Buffer PWB3.**

   Repeat as indicated. Discard flow-through.

10. **Elute the plasmid DNA with ▲25 ml ●100 ml Buffer PEB5.**

    We recommend precipitating the eluate as soon as possible (step 11). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.

    Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 13).

11. **Add ▲18 ml ●70 ml isopropanol (room-temperature) to precipitate the eluted plasmid DNA.**

    Mix carefully and centrifuge at ≥15,000 x g for 30 min at 4 °C. Carefully discard the supernatant.

12. **Add ▲7 ml ●10 ml 70 % ethanol (room-temperature) to wash the pellet.**

    Vortex briefly and centrifuge at ≥ 15,000 x g for 10 min at room temperature (18 – 25 °C).Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry 30 – 60 min 30 – 60 min at room temperature (18 – 25 °C) no less than the indicated time. Drying for longer periods of time will not harm the quality of plasmid DNA but overdrying may render the DNA less soluble.

13. **Dissolve pellet in an appropriate volume of buffer TE or sterile deionized water to reconstitute the DNA**

    Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).

Note: Determine plasmid yield by UV Spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

Protocols: Purification of low-copy plasmid DNA using SurePrep® Plasmid Mini, Midi Kit

- Please read Important Notes at Pg no. 5-7 before starting.
- Black (Marked with a ■) denotes values for SurePrep® Mini kit; Blue (marked with a ▲) denotes value for SurePrep® Plasmid Midi kit.

Procedure

1. **Pick a single colony from freshly streaked selective plate and inoculate the appropriate volume of LB medium containing the selective antibiotic. Incubate for approx. 12-16 hrs at 37°C with vigorous shaking.**

   Use a tube or flask with a volume of at least 4 times the volume of the culture

2. **Harvest the bacterial cells by centrifugation at 4500 - 6000 x g for 15 min at 4 °C.**

   If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

3. **Carefully resuspend the pellet of bacterial cells in ■0.8 ml ▲8.0 ml Buffer PRB1 + RNase A for cell lysis.**

4. **Add ■0.8 ml ▲8.0 ml Buffer PLB2 to the suspension.**

   Mix gently by inverting the tube 6 - 8 times. Incubate the mixture at room temperature (18 – 25 °C) for 2 – 3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

5. **Add ■0.8 ml ▲8.0 ml pre-cooled Buffer PNB3 (4 °C) to the suspension.**

   Immediately Mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. Incubate the suspension on ice for 5 min.

6. **Equilibrate a SurePrep® Mini, Midi Column with ■1 ml ▲2.5 ml Buffer PQB2.**

   Allow the column to empty by gravity flow. Discard flow-through.

7. **Clear the bacterial lysate by following either option 1 or option 2, described below.**
   This step is extremely important; excess precipitate left in suspension may clog the SurePrep® Column in later steps.
   Option 1. Place a SurePrep® Folded Filter in a small funnel for support, and prewet the filter with a few drops of Buffer PQB2 or sterile deionized water. Load the bacterial lysate onto the wet filter and collect the flowthrough.
   Note: Do not use SurePrep® Folded Filters with Mini columns.
   Option 2. Centrifuge at > 12,000 x g for the minimum time indicated 15 min 25 min at 4°C. If the suspension contains residual precipitate after the first centrifugation, either repeat this step or proceed with option 1.

8. **Load the cleared lysate from step 7 onto the SurePrep® Column. Allow the column to empty by gravity flow.**

9. **Wash the column with ■ 2 x 2 ml ▲ 12 ml Buffer PWB3.**
   Repeat as indicated. Discard flow-through.

10. **Elute the plasmid DNA with ■ 1 ml ▲ 5 ml Buffer PEB5.**
    Preheating Buffer PEB5 to 50 °C prior to elution may improve yields for high-molecular weight constructs such as BACs. We recommend precipitating the eluate as soon as possible (step 11). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.
    Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 13).

11. **Add ■ 0.75 ml ▲ 3.5 ml isopropanol (room-temperature) to precipitate the eluted plasmid DNA.** Mix carefully and centrifuge at ≥ 15,000 x g for 30 min at 4 °C. Carefully discard the supernatant.

12. **Add ■ 500 µl ▲ 2 ml 70 % ethanol (room-temperature) to wash the pellet.**
    Vortex briefly and centrifuge at ≥ 15,000 x g for 10 min at room temperature (18 – 25 °C). Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry for ■ 5 – 10 min ▲ 5 – 10 min at room temperature (18 – 25 °C).
    Drying for longer periods of time will not harm the quality of plasmid DNA but overdrying may render the DNA less soluble.

13. **Dissolve pellet in an appropriate volume of buffer TE or sterile deionized water to reconstitute the DNA**
    Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).

Note: Determine plasmid yield by UV Spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

Purification of low-copy plasmid DNA using SurePrep® Plasmid Maxi and Mega Kit

- Please read Important Notes at Pg no. 5-7 before starting.
- Blue (marked with a ▲) denotes value for SurePrep® Plasmid Maxi and Red (marked with a ●) denotes value for SurePrep® Plasmid Mega kit.

Procedure

1. **Pick a single colony from freshly streaked selective plate and inoculate the appropriate volume of LB medium containing the selective antibiotic. Incubate for approx. 12-16 hrs at 37°C with vigorous shaking.**
   Use a tube or flask with a volume of at least 4 times the volume of the culture

2. **Harvest the bacterial cells by centrifugation at 4500-6000 x g for 15 min at 4°C.**
   If you wish to stop the protocol and continue later, freeze the cell pellets at –20°C.

3. **Carefully resuspend the pellet of bacterial cells in ▲ 24 ml ● 90 ml Buffer PRB1 + RNase A.**

4. **Add ▲ 24 ml ● 90 ml Buffer PLB2 to the suspension.**
   Mix gently by inverting the tube 6 - 8 times. Incubate the mixture at room temperature (18 – 25 °C) for 2 – 3 min (max. 5 min). Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.

5. **Add ▲ 24 ml ● 90 ml pre-cooled Buffer PNB3 (4 °C) to the suspension.**
   Immediately mix the lysate gently by inverting the flask 6 – 8 times until a homogeneous suspension containing an off-white flocculate is formed. Incubate the suspension on ice for 5 min.

6. **Equilibrate SurePrep® Maxi and Mega Column with ▲ 6 ml ● 20 ml Buffer PQB2.**
   Allow the column to empty by gravity flow. Discard flow through.

7. **Clear the bacterial lysate by following either option 1 or option 2, described below. This step is extremely important; excess precipitate left in suspension may clog the SurePrep® Column in later steps.**
   Option 1. Place a SurePrep® Folded Filter in a large funnel for support, and prewet the filter with a few drops of Buffer PQB2 or sterile deionized water. Load the bacterial lysate onto the wet filter and collect the flow-through. Shortly spin down the lysate at low g-force in order to let the cellular debris settle before loading on the SurePrep® Folded Filter. When the centrifuge has stopped, carefully decant the partially cleared lysate onto the wet filter and collect the flow-through. For the Mega column alternatively the vacuum operated SurePrep® Bottle Top Filters (not included) can be used for filtration of the lysate.
   Option 2. Centrifuge at > 12,000 x g for the minimum time 40 min 50 min at 4 °C. If the suspension contains residual precipitate after the first centrifugation, either repeat this step or proceed with option 1.

8. **Load the cleared lysate from step 7 onto the SurePrep® Column. Allow the column to empty by gravity flow.**

9. **Wash the column with ▲ 2 x 18 ml ● 2 x 50 ml Buffer PWB3.**
   Repeat as indicated. Discard flow-through.

10. **Elute the plasmid DNA with ▲ 15 ml ● 25 ml Buffer PEB5.** Preheating Buffer PEB5 to 50 °C prior to elution may improve yields for high-molecular weight constructs.
    We recommend precipitating the eluate as soon as possible (step 11). Nevertheless, the eluate can be stored in closed vials on ice for several hours. In this case the eluate should be preheated to room temperature before the plasmid DNA is precipitated.
    Optional: Determine plasmid yield by UV spectrophotometry in order to adjust the desired concentration of DNA (step 13).

11. **Add ▲ 11 ml ● 18 ml isopropanol (room-temperature) to precipitate the eluted plasmid DNA.** Mix carefully and centrifuge at ≥ 15,000 x g for 30 min at 4 °C. Carefully discard the supernatant.

12. **Add ▲ 5 ml ● 7 ml 70 % ethanol (room-temperature) to wash the pellet.**
    Vortex briefly and centrifuge at ≥ 15,000 x g for 10 min at room temperature (18 – 25 °C).
    Carefully remove ethanol from the tube with a pipette tip. Allow the pellet to dry **10 – 20 min 30 – 60 min at room temperature (18 – 25 °C)** no less than the indicated time.
    Drying for longer periods of time will not harm the quality of plasmid DNA but overdrying may render the DNA less soluble.

13. **Dissolve pellet in an appropriate volume of buffer TE or sterile deionized water to reconstitute the DNA**
    Depending on the type of centrifugation tube, dissolve under constant spinning in a sufficient amount of buffer for 10 – 60 min (3D-shaker).

Note: Determine plasmid yield by UV Spectrophotometry. Confirm plasmid integrity by agarose gel electrophoresis.

TROUBLESHOOTING GUIDE

No or low plasmid DNA yield

Possible cause
- SDS- or other precipitates are present in the sample

Suggestion(s)
- Load the PRB1 / PLB2 / PNB3 lysate sample onto the SurePrep® Column immediately after finishing the initial lysis steps. SDS and cell debris are removed by filtration with SurePrep® Folded Filters / SurePrep® Bottle Top Filters but if the cleared lysate is stored on ice for a longer period, new precipitate may appear. If precipitate is visible, it is recommended to filter the lysate again immediately before loading it onto the SurePrep® Column.

Possible cause
- pH or salt concentrations of buffers are too high

Suggestion(s)
- Especially if the customer prepares additional buffer it is recommended to thoroughly check the pH of each buffer. Adjust pH or prepare new buffers if necessary.

Possible cause
- Sample / lysate is too viscous

Suggestion(s)
- Watch maximal volumes and pellet wet weights given in the Handbook. Otherwise, filtration of the lysate and flow rate of the column will be insufficient.

Possible cause
- Column overloaded with nucleic acids

Suggestion(s)
- Use a larger column or purify excess nucleic acids on a new column. Refer to the recommended culture volumes listed in the table at the beginning of each protocol.

Possible cause
- Plasmid did not propagate

Suggestion(s)
- Check plasmid content in the cleared lysate by precipitation of an aliquot. Use colonies from fresh plates for inoculation and add appropriate antibiotic concentration to plates and media.

Possible cause
- Alkaline lysis was inefficient

Suggestion(s)
- If culture volume or pellet weight is too high, alkaline lysis becomes inefficient. Refer to the recommended culture volumes.

No or low plasmid DNA yield

Possible cause

- Lysate incorrectly prepared

Suggestion(s)

- After storage below 20 °C, SDS in Buffer PLB2 may precipitate causing inefficient lysis. Check Buffer PLB2 for precipitates before use and preheat the bottle to 30 – 40 °C if necessary in order to redissolve SDS.

Column is Blocked

Possible cause

- Sample is too viscous

Suggestion(s)

- Do NOT attempt to purify lysate prepared from a culture volume larger than recommended for any given column size. Increasing culture volumes not only block the column but can also reduce yields due to inefficient lysis.

Possible cause

- Precipitates occur during storage

Suggestion(s)

- Check cleared lysate for precipitates, especially if the lysate was stored for a longer time before loading. If necessary, clear the lysate again by filtration.

Possible cause

- Lysate was not completely cleared

Suggestion(s)

- Centrifuge at higher speed for a longer period of time, or use additional SurePrep® Folded Filters to clear the lysate.

Cellular DNA or RNA contamination of plasmid DNA

Possible cause

- Lysis treatment was too harsh

Suggestion(s)

- Be sure not to incubate the lysate in Buffer PLB2 for more than 5 min.

Possible cause

- Overzealous mixing during lysis allowed genomic DNA to shear off into the lysis buffer

Suggestion(s)

- If the lysate is too viscous to mix properly or gently, reduce culture volumes.

Possible cause

- RNase digestion was inefficient

Suggestion(s)

- RNase was not added to Buffer PRB1 or stored too long. Add new RNase to Buffer PRB1.

No nucleic acid pellet formed after precipitation

Possible cause

- Pellet was lost

Suggestion(s)

- Handle the precipitate with care. Decant solutions carefully. Measure DNA yield in Buffer PEB5 in order to calculate the potential plasmid DNA that should be recovered after precipitation.

Possible cause

- Pellet did not resuspend in buffer

Suggestion(s)

- Again, handle the pellet with care. Especially, if the DNA was precipitated in a > 15 ml tube the "pellet" may be smeared over the wall of the tube. Dissolve DNA with an appropriate volume of TE buffer by rolling the tube for at least 30 min.

Possible cause

- Nucleic acid did not precipitate

Suggestion(s)

- Check volumes of precipitating solvent, making sure to use at least 0.7 volumes of isopropanol and centrifuge for longer periods of time.

Nucleic acid pellet does not resuspend in buffer

Possible cause

- Pellet was over dried

Suggestion(s)

- Try dissolving at higher temperatures for a longer period of time (e.g., 2 h at 37 °C or overnight at RT), best under constant spinning (3D-shaker).

Possible cause

- Residual salt or organic solvent in the pellet

Suggestion(s)

- Wash the pellet with additional low-viscosity organic solvent (70 % ethanol), or increase the resuspension buffer volume.

Nucleic acid pellet is opaque or white instead of clear and glassy

Possible cause

- Salt has co-precipitated with the pellet

Suggestion(s)

- Use room-temperature isopropanol and check isopropanol purity. Do not precipitate by allowing the eluate to drip directly from the column into a tube containing isopropanol. Add isopropanol only after eluate has been collected. Try resuspending the pellet in Buffer PQB2, and reload onto the SurePrep® Column. Be sure to wash the column several times with Buffer PQB2 before loading the redissolved pellet onto the column.

Purified plasmid does not perform well in subsequent reactions

Possible cause

- DNA is contaminated with cellular debris or genomic DNA due to inefficient lysis

Suggestion(s)

- Reduce the culture volume, or increase the amount of Buffer PRB1, PLB2, and PNB3 used during the lysis steps.

Possible cause

- DNA is degraded

Suggestion(s)

- Make sure that all equipment (pipettors, centrifuge tubes, etc.) are clean and nuclease-free. Make sure that the alkaline lysis step (i.e., the incubation of sample after addition of Buffer PLB2) does not proceed for longer than 5 min.

SurePrep® Folded Filters clog during filtration

Possible cause

- Culture volumes used are too large

Suggestion(s)

- Reduce the culture volume or increase the amount of Buffer PRB1, PLB2, and PNB3 used during the lysis steps.

Possible cause

- Incubation time too short

Suggestion(s)

- Make sure that PRB1 / PLB2 / PNB3 lysate was incubated according to the protocol.

APPENDIX - A

Plasmid DNA yield and Quality

Plasmid yield is measured by UV spectroscopy by using the following relationship: 1 OD at 260 nm (1 cm path length) is equivalent to 50 µg plasmid DNA / ml. Plasmid quality is checked initially by running a 1 % agarose gel. This will give information on the percentage of ccc form/structural integrity of isolated plasmid DNA. Plasmid quality is checked by UV spectroscopy (quotient A260 / A280). A value of 1.80 – 1.90 is an indication for pure plasmid DNA. Depending on further use of the purified plasmid, more sophisticated analytical methods may have to be applied for quantification of byproducts.

APPENDIX - B

Composition of Buffers

Resuspension Buffer PRB1	:	50 mM Tris-HCl, 10 mM EDTA, 100 µg / ml RNase A, pH 8.0
Lyis Buffer PLB2	:	200 mM NaOH, 1 % SDS
Neutralization Buffer PNB3	:	2.8 M KAc, pH 5.1
Equilibration Buffer PQB2	:	100 mM Tris, 15 % ethanol, 900 mM KCl, 0.15 % Triton X-100, adjusted to pH 6.3 with H3PO4
Wash Buffer PWB3	:	100 mM Tris, 15 % ethanol, 1.15 M KCl, adjusted to pH 6.3 with H3PO4
Elution Buffer PEB5	:	100 mM Tris, 15 % ethanol, 1 M KCl, adjusted to pH 8.5 with H3PO4

Note: Keep all buffers tightly capped.

The concentration of KCl required for eluting the desired nucleic acid is highly dependent on the pH value of the eluent. For this reason, pH values must be carefully controlled if the buffers have been prepared by the customer. A deviation of more than 0.1 pH unit from the given values may affect yields. If you are consistently experiencing reduced yields, check the pH of all buffers before continuing. Buffers should be adjusted with H3PO4 or KOH.

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

SurePrep® Plasmid Kits 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 SurePrep® Plasmid Kits 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.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of SurePrep® Plasmid Kits are tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

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