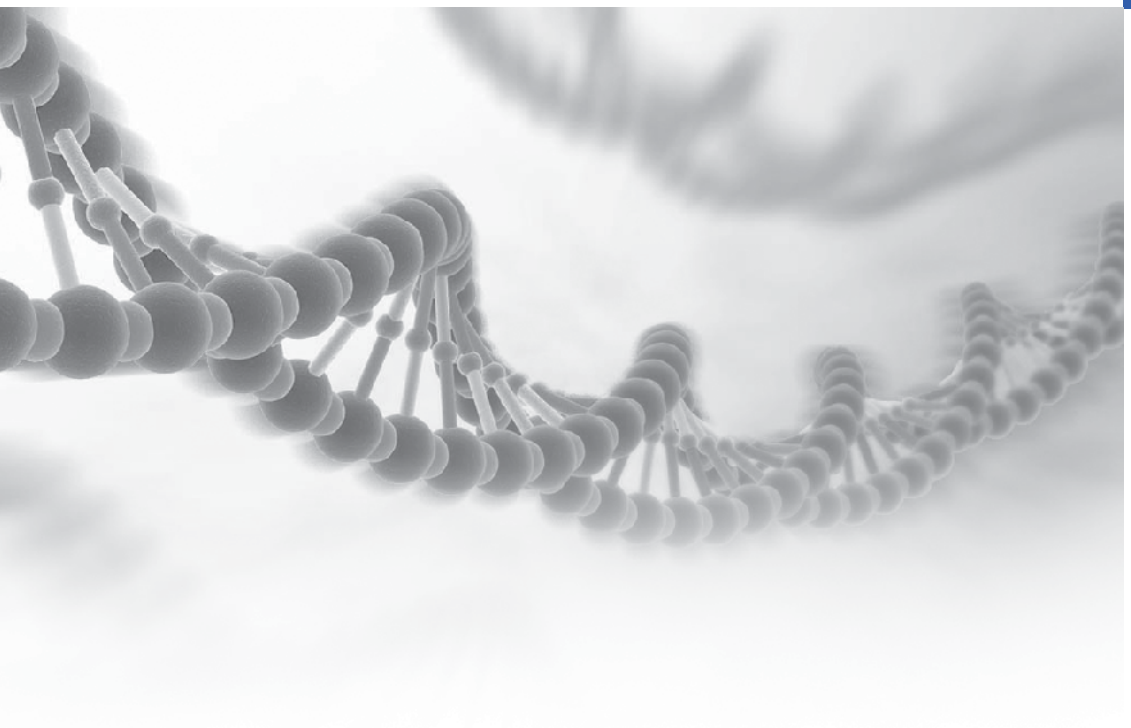
GENETIX BRAND

HANDBOOK

Nucleo-pore®

Purification of Transfection Grade Endotoxin Free Plasmid DNA

☐	**SurePrep® Plasmid Endofree Maxi Kit**	NP-15363	10 Preps
☐	**SurePrep® Plasmid Endofree Mega Kit**	NP-15365	5 Preps
☐	**SurePrep® Plasmid Endofree Giga Kit**	NP-15367	5 Preps



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Nucleo-pore®

Contents

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KIT CONTENTS

SurePrep®Plasmid Endofree Kit Pack Size	Maxi 10 Preps	Mega 5 Preps	Giga 5 Preps
Catalogues No.	NP-15363	NP-15365	NP-15367
SurePrep® Maxi/Mega/Giga columns	10	5	5
SurePrep® Folded Filters	10	-	-
SurePrep® Bottle Top Filters (Type1 or 2)	-	5 Type1	5 Type 2
Plastic washers	5	5	-
Resuspension Buffer RSB1-EF	150 ml	2 x 125 ml	3 x 250 ml
Lysis Buffer LB2-EF	150 ml	250 ml	750 ml
Neutralization Buffer NB3-EF	150 ml	250 ml	750 ml
Equilibration Buffer QB2-EF	100 ml	150 ml	600 ml
Wash Buffer WB3-EF	3 x 200 ml	2 x 400 ml	200 ml
			3x1000 ml
Wash Buffer WB4-EF	2 x 150 ml	400 ml	3 x 750 ml
Elution Buffer EB5-EF	200 ml	150 ml	600 ml
Redissolving Buffer RDB-EF	30 ml	30 ml	30 ml
NFW - EF (For 70% ethonal)	30 ml	30 ml	30 ml
NFWR - EF (for redissolving)	20 ml	30 ml	30 ml
RNase A (Lyophilized)	15mg	2x12mg	3x25mg
Handbook	1	1	1

STORAGE

SurePrep® Plasmid Endofree 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 RSB1-EF 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 Endofree Kits

Buffer LB2-EF

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

RNase A

Contains ribonuclease: sensitizer. Risk and safety phrases:* R 42/43 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 medical advice;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

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

Principle and Procedure

SurePrep® Plasmid Endofree kits employ a modified alkaline / SDS lysis procedure to prepare the bacterial cell pellet for plasmid purification. Both chromosomal and plasmid DNA are denatured under these alkaline conditions. Potassium acetate is then added to the denatured lysate, which causes the formation of a precipitate containing chromosomal DNA and other cellular compounds. The potassium acetate buffer also neutralizes the lysate. Plasmid DNA, which remains in solution, can revert to its native supercoiled structure. After equilibrating the appropriate SurePrep® Column with equilibration buffer, plasmid DNA is bound to the anion-exchange resin and finally eluted after efficient washing of the column. Endotoxins are removed by Buffer WB3-EF. After precipitation of the eluted DNA, it can easily be dissolved in Buffer RDB-EF or NFW-EF for further use.

Specifications of SurePrep® Plasmid Endofree kits

SurePrep® Plasmid Endofree kits contain SurePrep® Columns, appropriate buffers, and RNase A. Kits are available for the following column sizes: Maxi (500 µg Endofree Plasmid), Mega (2 mg Endofree Plasmid) and Giga (10 mg Endofree Plasmid).The protocols are suitable for purifying endotoxin-free plasmids with < 0.1 EU / µg. SurePrep® Columns are polypropylene columns containing SurePrep® Silica Resin packed between two inert filter elements. The columns are available in several sizes to accommodate a wide range of purification needs. All SurePrep® Columns are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of DNase and RNase. SurePrep® Resin can be used over a wide pH range, from pH 2.5 – 8.5, and can remain in contact with buffers for up to three hours without any change in its chromatographic properties. It remains intact in the presence of denaturing agents like formamide, urea, or common detergents such as Triton X-100 and NP-40.

Equipment and Reagents to Be Supplied by User

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

- Isopropanol (room-temperatured)
- 96 – 100% ethanol (room-temperatured)
- Ice
- 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)
- Refrigerated centrifuge capable of reaching ≥20,000 x g with rotor for the appropriate centrifuge tubes or bottles
- Centrifugation tubes or vessels with suitable capacity for the volumes specified in the respective protocol
- SurePrep® Rack Large or equivalent holder

IMPORTANT NOTES

It is highly recommended to read the handbook carefully before beginning the DNA Preparation. Experienced users who are using SurePrep® Plasmid purification kit for purification of High copy plasmids may refer to the Flow chart. The Flow chart is designed to be used as supplemented tool for quick reference during the purification procedure.

Bacterial Culture Growth

Yield and quality of plasmid DNA depend on, for example, 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. 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 cause decreased yield and plasmid quality. As a general rule, 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.

Culture Media

The cultivation of cells is recommended at 37 °C in LB (Luria-Bertani) medium at constant shaking (200 – 250 rpm). Alternatively, rich media like 2 x YT (Yeast / Tryptone) or TB (Terrific Broth) can 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. At least for Mega, Giga the use of an appropriate fermentation system is recommended in order to optimize cultivation conditions.

Culture Volume

Do not exceed the maximum recommended culture volumes as larger culture volumes will lead to an increase biomass and can affect the efficiency of the alkaline lysis, leading to reduced yield and purity of the preparation.

Kit	Recommended Culture Volume
SurePrep® Plasmid Endofree Maxi	30 -150 ml
SurePrep® Plasmid Endofree Maxi	150 - 500 ml
SurePrep® Plasmid Endofree Maxi	500 -2 ml

Difficult-to-lyse Bacterial strains

Isolate plasmid DNA from difficult-to-lyse strains by first resuspending the pellet in Buffer RSB1-EF containing lysozyme (2 mg / mL final concentration). Incubate at 37 °C for 30 minutes, then continue with the addition of Buffer LB2-EF, and proceed with the appropriate protocol.

Chloramphenicol amplification of low-copy plasmids

To dramatically increase the low copy number of pMB1 / colE1 derived plasmids grow the cell culture to mid or late log phase (OD600 ≈ 0.6 – 2.0) under selective conditions with an appropriate antibiotic. Then add 170 µg/mL chloramphenicol and continue incubation for a further 8 – 12 hours. Chloramphenicol inhibits host protein synthesis and thus prevents replication of the host chromosome. Plasmid replication, however, is independent of newly synthesized proteins and continues for several hours until up to 2000 – 3000 copies per cell are accumulated*.

Alternatively, the cell culture can be grown with only partial inhibition of protein synthesis under low chloramphenicol concentrations (10 – 20 µg/mL) resulting in a 5 – 10-fold greater yield of plasmid DNA**.

Both methods show the positive side effect of much less genomic DNA per plasmid, but they obviously work only with plasmids that do not carry the chloramphenicol resistance gene. Furthermore, the method is only effective with low copy number plasmids under stringent control (e.g., pBR322). All modern high copy number plasmids (e.g., pUC) are already under relaxed control due to mutations in the plasmid copy number control genes and show no significant additional increase in their copy number.

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

*** Frenkel L, Bremer H: Increased amplification of plasmids pBR322 and pBR327 by low concentrations of chloramphenicol, DNA (5), 539 – 544, 1986.*

Buffer Notes

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

- Dissolve the lyophilized RNase A by the addition of 1 ml of Resuspension Buffer RSB1-EF. Wearing gloves is recommended. Pipette up and down until the RNase A is completely dissolved. Transfer the RNase A solution back to the bottle containing Buffer RSB1-EF and shake well. Indicate date of RNase A addition. The final concentration of RNase A is 100 µg / mL Buffer RSB1-EF. Store Buffer RSB1-EF with RNase A at 4°C. The solution will be stable at this temperature for at least 6 months.
- Lysis Buffer LB2-EF should be stored at room temperature (18 – 25 °C) since the containing SDS may precipitate at temperatures below 18 °C. If precipitation occurs, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is dissolved.
- Pre-cool Neutralization Buffer NB3-EF to 4 °C.
- In order to prepare "endotoxin-free 70 % ethanol", add 70 ml of ethanol (96 – 100 %) to each 30 ml bottle containing NFW-EF in SurePrep® Plasmid Endofree Maxi, Mega and Giga kit.

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 Maxi, Mega, and Giga are at least 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

- Cell pellets can easily be stored for several months at - 20 °C.
- Cleared lysates can be kept on ice or at 4 °C for several days.
- For optimal performance the column purification should not be interrupted. However, the columns can be left unattended for several hours since the columns do not run dry. This might cause only small losses in DNA yield.
- The eluate can be stored for several days at 4 °C. Note that the eluate should be warmed up to room temperature before precipitating the DNA to avoid co-precipitation of salt.

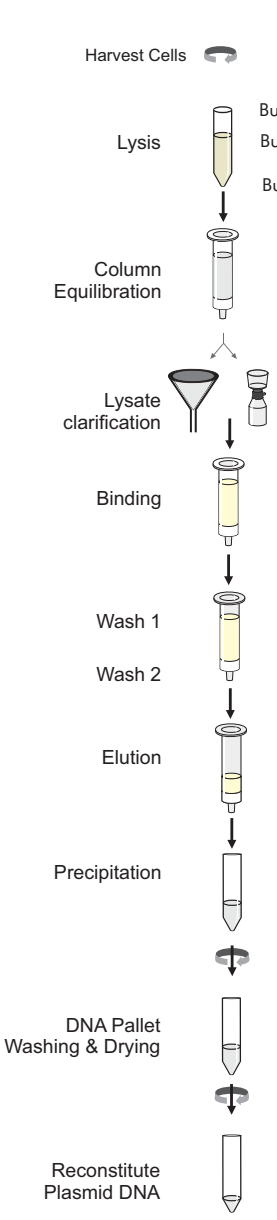
Lysate Filtration

After alkaline lysis, the solution has to be clarified from, for example, the cell debris through the supplied SurePrep® Folded Filters or SurePrep® Bottle Top Filters in order to prevent clogging of the column. For the SurePrep® Mega and Giga Column, use the supplied vacuum operated SurePrep® Bottle Top Filters for filtration of the lysate. The bottle top filters make the separation of the bacterial lysate and SDS precipitate easy, quick, and convenient. Adjust the bottle top filter to a suitable flask (e.g., Schott), load the bacterial lysate, and apply the vacuum. After 5 min the solution will have passed through. Load the resulting clear lysate onto the corresponding SurePrep® Column and discard the bottle top filter. For the SurePrep® Midi Column use the supplied SurePrep® Folded Filters 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 precipitate cellular debris from plasmid samples. For correct use please follow the instructions given in step 7 of the corresponding protocol.

Elution Method

Elution is carried out into a new tube with the volume of elution buffer indicated in the corresponding protocol. The plasmid DNA is precipitated by 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. Only use room-temperature (18 – 25 °C) isopropanol to prevent spontaneous co-precipitation of salt.

SurePrep® Endofree Plasmid Kits (Maxi, Mega, Giga)



	MAXI	MEGA	GIGA
Harvest 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
Lysis Buffer RSB1-EF	12 mL	40 mL	120 mL
Buffer LB2-EF	12 mL < 5 min at RT	40 mL < 5 min at RT	120 mL < 5 min at RT
Buffer NB3-EF	12 mL 5 min at 0 °C	40 mL /	120 mL /
Column Equilibration	Buffer QB2-EF 5 mL	Buffer QB2-EF 25 mL	Buffer QB2-EF 100 mL
Lysate clarification	Folded Filter 20 min	Bottle Top Filter Type 1 5 min	Bottle Top Filter Type 2 5 min
Binding	Load cleared lysate onto the column	Load cleared lysate onto the column	Load cleared lysate onto the column
Wash 1	Buffer WB3-EF 2 x 24 mL	Buffer WB3-EF 1 x 60 mL 2 x 40 mL	Buffer WB3-EF 4 x 150 mL
Wash 2	Buffer WB4-EF 2 x 12 mL	Buffer WB4-EF 60 mL	Buffer WB4-EF 3 x 130 mL
Elution	Buffer EB5-EF 15 mL	Buffer EB5-EF 25 mL	Buffer EB5-EF 100 mL
Precipitation	Isopropanol 11 mL	Isopropanol 18 mL	Isopropanol 70 mL
	≥ 15,000 x g 30 min at 12 °C	≥ 15,000 x g 30 min at 12 °C	≥ 15,000 x g 30 min at 12 °C
DNA Pallet Washing & Drying	70 % ethanol 5 mL	70 % ethanol 7 mL	70 % ethanol 10 mL
	≥ 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 RDB-EF or NFWR-EF	Appropriate volume of RDB-EF or NFWR-EF	Appropriate volume of RDB-EF or NFWR-EF

SurePrep® Plasmid Endofree plasmid purification (Maxi, Mega, Giga)

- Please read Important Notes at pg No…..before starting
- Blue (marked with a ▲) denotes values for the SurePrep® Plasmid Endofree Maxi Kit; red (marked with a ●) denotes values for the SurePrep® Plasmid Endofree Mega Kit and Black (marked with a ■) denotes value for the SurePrep® Plasmid Endofree Giga Kit.

Procedure

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

2. **Harvest bacteria from 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 ▲12 ml, ● 40 ml, ■120 ml Buffer RSB1-EF + RNase A to lyse the cells.**

4. **Add ▲12 ml, ● 40 ml, ■120 ml Buffer LB2-EF 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 ▲ 12 ml, ● 40 ml, ■ 120 ml Buffer NB3-EF (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.
   Endofree Maxi : Incubate the suspension for 5 min on ice before continuing with step 7, "Clarification of the lysate".
   Endofree Mega and Giga: Pour the lysate immediately into the SurePrep® Bottle Top Filter Type 1(Mega) or Type 2 (Giga) and continue as described in step 7 of this protocol.
   To save time, the equilibration of the SurePrep® Columns (see step 6) can be started during the clarification of the lysate as described in step 7 .

6. Equilibrate a SurePrep® Maxi, Mega or Giga Column with ▲ 5 ml, ● 25 ml, ■ 100 ml Buffer QB2-EF. Allow the column to empty by gravity flow. Discard flow-through.

7. **Clear the bacterial lysate by using SurePrep® Folded Filters (Maxi Endofree) or SurePrep® Bottle Top Filters (Mega /Giga Endofree).**
   This step is extremely important; excess precipitate left in suspension may clog the SurePrep® Column in later steps.
   SurePrep® Plasmid Endofree Maxi: Place the SurePrep® Folded Filter in a small funnel for support, and pre-wet the filter with a few drops of Buffer QB2-EF or sterile deionized H2O. Load the bacterial lysate onto the wet filter and collect the flow-through.
   SurePrep® Plasmid Endofree Mega and Giga: Pour the lysate immediately into the SurePrep® Bottle Top Filter Type 1 (Mega) or Type 2 (Giga) and incubate at room temperature for 10 min. Switch on the vacuum source (optimal - 0.4 to - 0.6 bar) in order to filtrate the lysate through the SurePrep® Bottle Top Filter. After all liquid has passed the filter (3 – 5 min) switch off the vacuum source.
   SurePrep® Plasmid Endofree Giga: It is possible to stir the precipitate with a spatula gently onto the filter ▲20 min, ● 5 min, ■ 5 min. In order to recover residual liquid, switch on vacuum again for another minute.
   Alternatively: Centrifuge the crude lysate at high speed (> 12,000 x g) at 4°C for ▲40 min (Maxi), 50 min (Mega) and 60 min (Giga). Subsequently after centrifugation, carefully remove the supernatant from the white precipitate and load it onto the equilibrated SurePrep® Column.

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 24 ml ●1 x 60 ml ■4 x 150 ml Buffer WB3-EF.
   2 x 40 ml
   Repeat as indicated. Discard flowthrough.

10. Wash the column with ▲ 2 x 12 ml ● 1 x 60 ml ■3 x 130 ml Buffer WB4-EF. Repeat as indicated. Discard flow-through.

11. Elute the plasmid DNA with ▲ 15 ml ●25 ml ■100 ml Buffer EB5-EF.
    We recommend precipitating the eluate as soon as possible (step 12). 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 14).

12. Add room-temperature ▲ 11 ml ● 18 ml ■ 70 ml isopropanol to precipitate the eluted plasmid DNA. Mix carefully and centrifuge at ≥15,000 x g for 30 min at 12 °C. Carefully discard the supernatant.

13. **Add room-temperature ▲ 5 ml ● 7 ml ■ 10 ml endotoxin-free 70 % ethanol** to the pellet. Vortex briefly and centrifuge at ≥ 15,000 x g for 10 min at room temperature (18 – 25°C). For preparation of endotoxin-free 70% ethanol refer to Important Notes. 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 ●30 – 60 min ■30 – 60 min.
    Drying for longer periods of time will not harm the quality of plasmid DNA but overdrying may render the DNA less soluble.

14. Dissolve pellet in an appropriate volume of Buffer RDB-EF or NFWR-EF 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 RSB1-EF / LB2-EF / NB3-EF 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
- Sample / lysate is too viscous

Suggestion(s)
- Watch maximal volumes and pellet wet weights given in the manual. 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.

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.

Possible cause
- Lysate incorrectly prepared

Suggestion(s)
- After storage below 20 °C, SDS in Buffer LB2-EF may precipitate causing inefficient lysis. Check Buffer LB2-EF for precipitates before use and prewarm 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)
- 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 LB2-EF 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 RSB1-EF or stored too long. Add new RNase to Buffer RSB1-EF.

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 EB5-EF in order to calculate the potential plasmid DNA that should be recovered after precipitation.

Possible cause
- Pellet was smeared over the tube wall

Suggestion(s)
- Dissolve DNA with an appropriate volume of Buffer RDB-EF 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 endotoxin-free 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. Centrifuge at 12 °C. Try resuspending the pellet in Buffer QB2-EF, and repeat the precipitation step.

Purified plasmid does not perform well in downstream applications

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 RSB1-EF, LB2-EF, and NB3-EF 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 LB- EF) 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 RSB1-EF, LB2-EF, and NB3-EF used during the lysis steps.

Possible cause

- Incubation time too short

Suggestion(s)

- Make sure that RSB1-EF / LB2-EF / NB3-EF lysate was incubated according to the protocol.

Appendix : Bacterial Endotoxins Removal

What are endotoxins?

Endotoxins, also known as lipopolysaccharides or LPS, are cell membrane components of Gram-negative bacteria (e.g., *E. coli*). The lipid portion of the outer layer of the outer membrane is completely composed of endotoxin molecules. A single *E. coli* cell contains about 2 million LPS molecules, each consisting of a hydrophobic lipid A moiety, a complex array of sugar residues and negatively charged phosphate groups. Therefore, each endotoxin molecule possesses hydrophobic, hydrophilic, and charged regions giving it unique features with respect to possible interactions with other molecules. Bacteria shed small amounts of endotoxins into their surroundings while they are actively growing and large amounts when they die. During lysis of bacterial cells for plasmid preparations, endotoxin molecules are released from the outer membrane into the lysate.

Endotoxin contamination of different plasmid preparation methods

The chemical structure and properties of endotoxin molecules and their tendency to form micellar structures lead to copurification of endotoxins with plasmid DNA. For example, in CsCl ultracentrifugation, the CsCl-banded DNA is easily contaminated with endotoxin molecules, which have a similar density in CsCl to plasmid–ethidium bromide complexes.

On size exclusion resins, the large size of the micellar form of endotoxin causes the molecule to behave like a large DNA molecule; and in anion-exchange chromatography, the negative charges present on the endotoxin molecule can interact with anion-exchange resins, thus leading to copurification of endotoxins with the plasmid DNA. However, the level of endotoxin contamination found in plasmid DNA is dependent on the purification method used. SurePrep® Plasmid Endofree Kits and 2 x CsCl gradientcentrifugation both yield very pure DNA with relatively low levels of endotoxoin. Silicaslurry- purified DNA contains significantly higher endotoxin contamination. DNApurified with EndoFree Plasmid Kits contains only negligible amounts of endotoxin (<0.1 EU/µg plasmid DNA)

How are endotoxins measured?

Historically, endotoxins were measured in a clotting reaction between the endotoxin and a clottable protein in the amoebocytes of Limulus polyphemus, the horseshoe crab. Today much more sensitive photometric tests (e.g., Kinetic-QCL Test from BioWhittaker, Inc.) are used, which are based on a Limulus amoebocyte lysate (LAL) and a synthetic color-producing substrate. LPS contamination is usually expressed in endotoxin units (EU). Typically, 1 ng LPS corresponds to 1–10 EU.

Influence of endotoxins on biological applications

Endotoxins strongly influence transfection of DNA into primary cells and sensitive cultured cells, and increased endotoxin levels lead to sharply reduced transfection efficiencies. Furthermore, it is extremely important to use endotoxin-free plasmid DNA for gene therapy applications, since endotoxins cause fever, endotoxic shock syndrome, and activation of the complement cascade in animals and humans. Endotoxins also interfere with *in vitro* transfection into immune cells such as macrophages and B cells by causing nonspecific activation of immune responses. These responses include the induced synthesis of immune mediators such as IL-1 and prostaglandin. It is important to make sure that plasticware, media, sera, and plasmid DNA are free of LPS contamination in order to avoid misinterpretation of experimental results.

Removal of endotoxins

The EndoFree Plasmid procedure integrates endotoxin removal into the standard SurePrep® Plasmid Endofree purification procedure. The neutralized bacterial lysate is filtered through a SurePrep® Folded Filters or Bottle Top Filters and incubated on ice with a specific endotoxin removal buffer (Buffer WB3-EF). The endotoxin removal buffer prevents LPS molecules from binding to the resin in the SurePrep allowing purification of DNA containing less than 0.1 endotoxin units per µg plasmid DNA.

Endotoxin-free plasticware and glassware

In order to avoid recontamination of plasmid DNA after initial endotoxin removal, we recommend using only new plasticware which is certified to be pyrogen- or endotoxin free. Endotoxin-free or pyrogen-free plasticware can be obtained from many different suppliers. Endotoxins adhere strongly to glassware and are difficult to remove completely during washing. Standard laboratory autoclaving procedures have little or no effect on endotoxin levels. Moreover, if the autoclave has previously been used for bacteria, the glassware will become extensively contaminated with endotoxin molecules. Heating glassware at 180°C overnight is recommended to destroy any attached endotoxin molecules.

It is also important not to recontaminate the purified endotoxin-free DNA by using reagents that are not endotoxin-free. All buffers supplied with the EndoFree Plasmid Kits are tested and certified to be endotoxin-free, as are the water for preparation of 70% ethanol and the TE buffer for resuspension.

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® Endofree 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® Endofree 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® Endofree 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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