For life science research only.
Not for use in diagnostic procedures.

Print Protocol

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Genopure Plasmid Midi Kit

Version 11

Content version: July 2017

For the isolation of plasmid DNA from bacterial cultures

Cat. No. 03 143 414 001

Kit for 20 isolations from 5 to 30 ml bacterial culture volume of high copy number plasmids

Store the kit at +15 to +25°C

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Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests

20 plasmid midi preparations, up to eight samples can be processed at a time.

⚠ The stated number of isolations is valid for the isolation of high copy number plasmids.

When isolating P1 constructs or other low copy plasmids using alkaline lysis protocols, incomplete bacterial lysis is the number one problem resulting in low yield. To improve alkaline lysis when isolating low copy number plasmid, the Genopure Buffer Set for Low Copy Number Plasmids as a supplement to the Genopure Plasmid Kits. The Genopure Buffer Set for Low Copy Number Plasmids is available as Cat. No. 04 634 772 001.

Kit Contents

⚠ All solutions are clear. Before using the Lysis Buffer and the Neutralization Buffer, always check them for precipitates. Do not use any solution that contains precipitates. Instead, warm it at ambient temperature (>+20°C) [Neutralization Buffer] or in a +37°C water bath [Neutralization or Lysis Buffer] until the precipitates have dissolved. Mix solution well before use.

Vial/ Cap	Label	Contents/Function
1 colorless	Suspension Buffer	• 100 ml • for suspension of bacterial cell pellets
2 blue	RNase A	• 12 mg • to be dissolved in Suspension Buffer
3 green	Lysis Buffer	• 100 ml • for bacterial cell lysis ⚠ SDS in the Lysis Buffer may crystallize at temperatures <+20°C. If crystals have formed, dissolve them as described in the Note above.
4 white	Neutralization Buffer	• 100 ml • to form a stable cellular debris precipitate
5 red	Equilibration Buffer	• 70 ml • for equilibrating the columns prior to use
6 blue	Wash Buffer	• 2 × 125 ml • for removal of residual impurities
7 yellow	Elution buffer	• 125 ml • for plasmid elution
8	NucleoBond AX 100 Columns	• 20 columns • for the isolation step

Vial/ Cap	Label	Contents/Function
9	Folded filters	• 20 filters • to eliminate a centrifugation step • to remove cellular debris
10	Sealing rings	• 10 rings • to fix the columns in test tubes

Storage and Stability

The Genopure Plasmid Midi Kit components must be stored at +15 to +25°C. If properly stored, all kit components are stable until the expiration date printed on the label. The kit is shipped at ambient temperature.

⚠ After adding RNase A to the Suspension Buffer, store the buffer at +2 to +8°C, where it will be stable for 6 months.

⚠ Do not freeze the columns. Do not expose the kit component to extended heat for longer periods of time. Keep all buffer bottles tightly capped to ensure the stability of the pH values needed.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform plasmid isolations include:

- Centrifuge and tubes for harvesting bacterial cultures, capable of ⩾15,000 × *g*
- Isopropanol
- 70% ethanol
- TE buffer or other low salt buffer
- Tube for collecting and precipitating eluted plasmid DNA
- Funnel for clearing of lysates by folded filters

Application

The Genopure Plasmid Midi Kit is used to prepare purified plasmid DNA in medium quantities known as "midi preps". Using a modified alkaline lysis method, highly purified plasmid DNA from *E. coli* (free of RNA contamination according to current quality control procedures) is generated.

The kit is designed for the isolation of up to 100 µg .plasmid DNA from bacterial culture. Depending on the copy number of the plasmids use either 5-ml to 30-ml (high-copy number) or 10-ml to 100-ml (low-copy number) bacterial suspension.

Plasmid DNA isolated with this kit is suitable for all molecular biology applications, *e.g.*, transfection, PCR, restriction analysis, Southern blotting, sequencing and cloning.

Assay Time

Total time	60 min including a filtration step after alkaline lysis
Hands-on time	Minimal hands-on time required (about 10 min)

2. How To Use this Product

2.1 Before You Begin

Sample Material

- 5 – 30 ml *E. coli* culture, transformed with a high copy number plasmid.
- 10 – 100 ml *E. coli* culture, transformed with a low copy number plasmid

⚠ Harvest cultures at a density between 2.0 and 6.0 A_{600} units per ml bacterial culture.

Media

The isolation method is optimized for cultures grown in LB media; other rich media may require increased volumes of Suspension-, Lysis- and Neutralization Buffer, and an additional wash step.

Plasmid Size

The isolation procedure is suitable for all plasmid sizes; lysates of larger constructs (up to 100 kb) should be cleared by filtration to avoid shearing.

Preparation of overnight culture

Transfer a single bacterial colony from a selective plate. Inoculate a starter culture in 1 - 5 ml LB medium containing the proper antibiotic in an appropriately sized, loosely capped tube. Incubate the culture at +37°C overnight (12 - 16 hours) with vigorous shaking (220 – 250 rpm). 30 - 100 ml selective LB medium is inoculated with 1 ml of the overnight starter culture. Incubate at +37°C overnight (12 - 16 hours) with vigorous shaking (220 - 250 rpm). Typically the cell density is approximately 2 - 6 A_{600} units.

⚠ Higher culture volumes can cause overloading of the column resulting in lower yields due to inefficient cell lysis and clogging of the column.

Preparation of Working Solutions

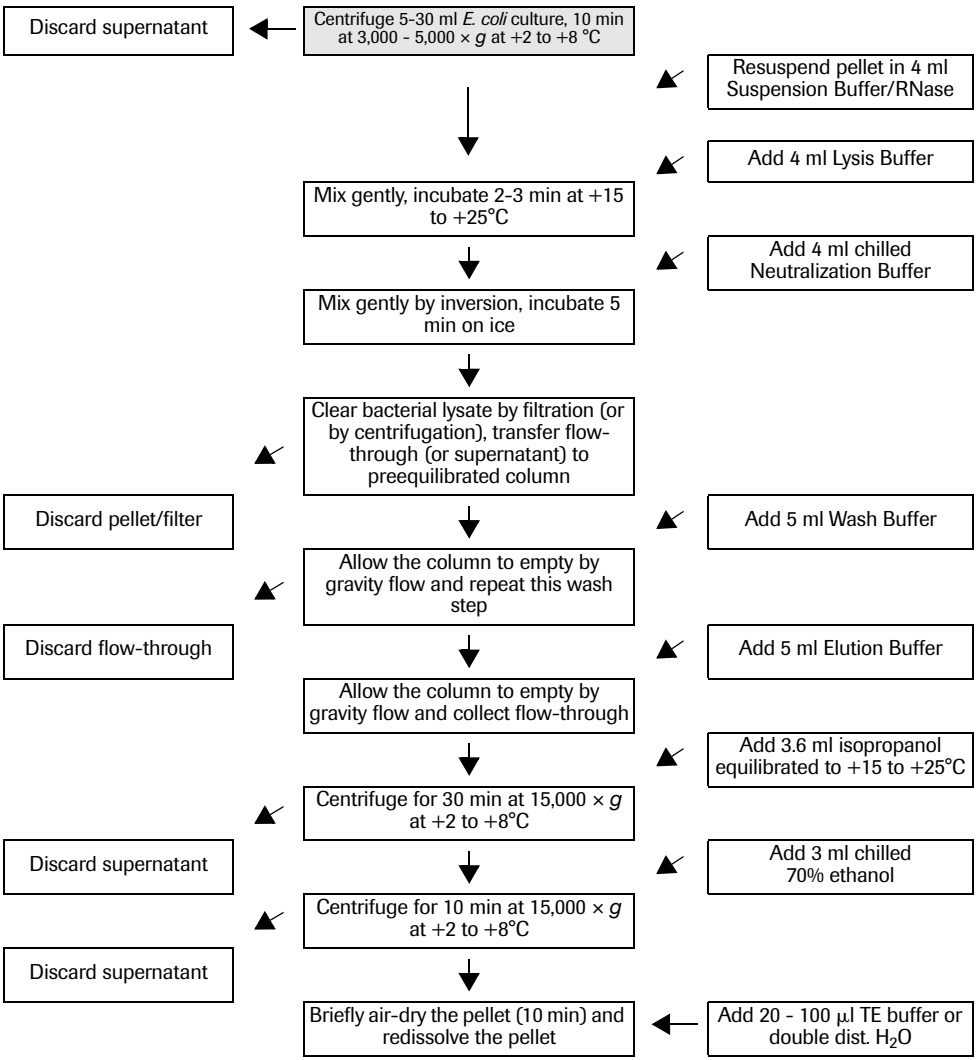
In addition to the ready-to-use buffers supplied in the kit, the following working solution needs to be prepared.

Suspension Buffer/RNase A

To dissolve the lyophilized enzyme in Suspension Buffer, pipet 1 ml of Suspension Buffer (bottle 1, colorless cap) into the glass vial containing the lyophilized RNase A (bottle 2, blue cap). Reinsert the rubber stopper and invert the vial until all lyophilizate (including any that might stick to the rubber stopper) is dissolved. Transfer the dissolved enzyme back to the Suspension Buffer bottle (bottle 1). This is sufficient working solution for 60 Midi preps (isolation of up to 100 µg plasmid DNA/preparation).

When preparing aliquots of the working solution, the final concentration of RNase A in the working solution must be 100 µg/ml.

Reconstituted buffer is stable for 6 months, if stored at+2 to +8°C.


Centrifuge 5-30 ml *E. coli* culture, 10 min at 3,000 - 5,000 × *g* at +2 to +8 °C
Discard supernatant
Resuspend pellet in 4 ml Suspension Buffer/RNase
Add 4 ml Lysis Buffer
Mix gently, incubate 2-3 min at +15 to +25°C
Add 4 ml chilled Neutralization Buffer
Mix gently by inversion, incubate 5 min on ice
Clear bacterial lysate by filtration (or by centrifugation), transfer flow-through (or supernatant) to preequilibrated column
Discard pellet/filter
Add 5 ml Wash Buffer
Allow the column to empty by gravity flow and repeat this wash step
Discard flow-through
Add 5 ml Elution Buffer
Allow the column to empty by gravity flow and collect flow-through
Add 3.6 ml isopropanol equilibrated to +15 to +25°C
Centrifuge for 30 min at 15,000 × *g* at +2 to +8°C
Discard supernatant
Add 3 ml chilled 70% ethanol
Centrifuge for 10 min at 15,000 × *g* at +2 to +8°C
Discard supernatant
Briefly air-dry the pellet (10 min) and redissolve the pellet
Add 20 - 100 µl TE buffer or double dist. H_2O

❶ • Centrifuge bacterial cells from 5 – 30 ml culture grown in LB medium (5 – 10 min / 3,000 – 5,000 × *g* / +2 to +8°C).
• Discard the supernatant.
• Carefully resuspend the pellet in 4 ml Suspension Buffer + RNase and mix well.

❷ Add 4 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times. Incubate 2 – 3 min at +15 to +25°C.

⚠ To avoid shearing genomic DNA, do not vortex the suspension in Lysis Buffer. To prevent release of chromosomal DNA from the cell debris, do not incubate for more than 5 min.

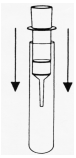
❸ • Add 4 ml chilled Neutralization Buffer to the suspension.
• Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogenous suspension is formed.
• Incubate the tube 5 min on ice.

🔍 The solution becomes cloudy and a flocculent precipitate will form.

❹ Clear the lysate by either centrifugation (4a) or by filtration (4b).
4a. Centrifuge at high speed (>30 min / >12,000 × *g* / +2 to +8°C). Directly after centrifugation, carefully remove the supernatant from the white precipitate and proceed with step 5.
4b. Put a folded filter into a funnel that has been inserted into a 50 ml plastic tube.
• Moisten the filter with a few drops of Equilibration Buffer or double-distilled water.
• Load the lysate onto the wet, folded filter and collect the flow through.

⚠ The SDS precipitates with cellular debris when Neutralization Buffer is added; this white precipitate should not be loaded onto the column since it will clog the column. If the solution obtained after step 4 is not clear, remove the remaining precipitate by passing the solution over a folded filter.

❺ • Mount the sealing ring to the column as shown in the figure to fix the column in the collection tube.
• Insert one column into one collection tube.
• Equilibrate the column with 2.5 ml Equilibration Buffer.
• Allow the column to empty by gravity flow.
• Discard the flow through.



❻ • Load the cleared lysate from step 4 onto the equilibrated column.
• Allow the column to empty by gravity flow.
• Discard the flow through.

❼ • Wash the column with 5 ml Wash Buffer.
• Allow the column to empty by gravity flow.
• Discard the flow through.

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❽	• Repeat step 7. • Discard flow through and collection tube.
❾	• Re-insert the column into a new collection tube capable of withstanding high-speed centrifugation (≥15,000 × *g*). • Elute the plasmid with 5 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • The collected flow-through contains the plasmid.
❿	• Precipitate the eluted plasmid DNA with 3.6 ml isopropanol (equilibrated to +15 to +25°C). • Centrifuge immediately (30 min / ≥15,000 × *g* / +2 to+ 8°C). • Carefully discard the supernatant.
⓫	• Wash the plasmid DNA with 3 ml chilled (+2 to +8°C) 70% ethanol. • Centrifuge (10 min / >15,000 × *g* / +2 to +8°C). • Carefully remove ethanol from the tube with a pipette tip. • Air-dry the plasmid DNA pellet for 10 min.
⓬	Carefully redissolve the plasmid DNA pellet in 20 - 100 µl TE buffer or sterile double-distilled water.

Storage of sample

If you want to….	THEN...
Continue experiments	Use the purified plasmid DNA right away.
Analyse plasmid DNA	Store plasmid DNA at +2 to +8°C or –15 to -25°C for later analysis.

❶ • Centrifuge bacterial cells from 10 – 100 ml *E.coli* culture grown in LB medium (5 – 10 min / 3,000 – 5,000 × *g* / +2 to +8°C).
• Discard the supernatant.
• Carefully resuspend the pellet in 8 ml Suspension Buffer + RNase and mix well.

❷ Add 8 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times. Incubate 2 – 3 min at +15 to +25°C.

⚠ To avoid shearing genomic DNA, do not vortex the suspension in Lysis Buffer. To prevent release of chromosomal DNA from the cell debris, do not incubate for more than 5 min.

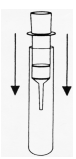
❸ • Add 8 ml chilled Neutralization Buffer to the suspension.
• Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogenous suspension is formed.
• Incubate the tube 5 min on ice.

🔍 The solution becomes cloudy and a flocculent precipitate will form.

❹ Clear the lysate by either centrifugation (4a) or by filtration (4b).
4a. Centrifuge at high speed (>30 min / >12,000 × *g* / +2 to +8°C). Directly after centrifugation, carefully remove the supernatant from the white precipitate and proceed with step 5.
4b. Put a folded filter into a funnel that has been inserted into a 50 ml plastic tube.
• Moisten the filter with a few drops of Equilibration Buffer or double-distilled water.
• Load the lysate onto the wet, folded filter and collect the flow-through.

⚠ The SDS precipitates with cellular debris when Neutralization Buffer is added; this white precipitate should not be loaded onto the column since it will clog the column. If the solution obtained after step 4 is not clear, remove the remaining precipitate by passing the solution over a folded filter.

❺ • Mount the sealing ring to the column as shown in the figure to fix the column in the collection tube.
• Insert one column into one collection tube.
• Equilibrate the column with 2.5 ml Equilibration Buffer.
• Allow the column to empty by gravity flow.
• Discard the flow through.



❻ • Load the cleared lysate from step 4 onto the equilibrated column.
• Allow the column to empty by gravity flow.
• Collect the flow through and load it a second time onto the column.
• Allow the column to empty by gravity flow.
• Discard the flow through.

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❼	• Wash the column with 4 ml Wash Buffer. • Allow the column to empty by gravity flow. • Discard the flow through.
❽	Repeat step 7.
❾	• Repeat step 7. • Discard flow through and collection tube.
❿	• Re-insert the column into a new collection tube capable of withstanding high-speed centrifugation ($\geq$15,000 × *g*). • Elute the plasmid with 2.5 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • Collect the flow through.
⓫	• Elute a second time with 2.5 ml prewarmed Elution Buffer (+50°C) and combine eluates. • Allow the column to empty by gravity flow. • The collected flow-through contains the plasmid DNA.
⓬	• Precipitate the eluted plasmid DNA with 3.6 ml isopropanol (equilibrated to +15 to +25°C). • Centrifuge immediately (30 min / $\geq$15,000 × *g* /+2 to +8°C). • Carefully discard the supernatant.
⓭	• Wash the plasmid DNA with 3 ml chilled (+2 to +8°C) 70% ethanol. • Centrifuge (10 min / >15,000 × *g* / +2 to +8°C). • Carefully remove ethanol from the tube with a pipette tip. • Air-dry the plasmid DNA pellet for 10 min.
⓮	Carefully redissolve the plasmid DNA pellet in 20 - 50 µl TE buffer or sterile double-distilled water.

Storage of sample

If you want to….	Then...
Continue experiments	Use the purified plasmid DNA right away.
Analyse plasmid DNA	Store plasmid DNA at +2 to +8 °C or –15 to -25°C for later analysis.

3. Results

Purity

Plasmid DNA is free of all other bacterial components, including RNA, according to the current quality control procedures (see section 5).

Yield

Depending on the *E. coli* strain and density of cell culture. Comparable to traditional purification methods.

The yield of plasmid DNA preparations depends on several parameters, including quality of the bacterial culture growth, amount of culture suspension used for the preparation, kind of plasmid used *etc.* As a rule of thumb, the typical yield of a high copy number plasmid is about 3 - 5 μg of DNA per ml of original bacterial culture (pUC, pTZ, pGEM in common host strains as XL-1 blue, HB101, JM 109). The typical yield of low copy number plasmids is about 0.2 - 1 μg of DNA per ml of original bacterial culture.

Application

Plasmid DNA isolated with this kit is suitable for all molecular biology applications, including transfection, PCR, restriction analysis, Southern blotting, sequencing and cloning.

	Possible Cause	Recommendation
Low nucleic acid yield or purity	Kit stored under non-optimal conditions.	Store kit at +15 to +25°C at all times.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	• Store all buffers at +15 to +25°C. • After adding RNase to Suspension Buffer store aliquots of the working solution at +2 to +8°C. • Close all reagent bottles tightly after each use to ensure stability, correct pH and freedom from contamination.
	Reagents and samples not completely mixed.	• Always mix the sample tube well after addition of each reagent. • Ensure Lysis Buffer and Neutralization Buffer are free of precipitates.
Low recovery of nucleic acids after elution	Wrong reagent has been used for elution. Salt is required for optimal elution.	Use the Elution Buffer from the kit.
Low plasmid yield	Too few cells in starting material.	Grow *E. coli* to an absorbance (A_{600}) of 2 - 6 before harvest.
	Incomplete cell lysis.	• Be sure the *E. coli* pellet is completely resuspended in Suspension Buffer. • Make sure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer). • Make sure a cloudy white precipitate forms when Neutralization Buffer is added to the lysate.
	Lysate did not bind completely to column.	Pre-equilibrate the column by adding Equilibration Buffer before adding sample.
RNA present in final product	RNase not completely dissolved.	To reconstitute the lyophilized RNase completely: 1. Pipet 1 ml of Suspension Buffer into the glass vial containing lyophilized RNase. 2. Stopper and invert the vial until all the lyophilizate (including product on the rubber stopper) is dissolved. 3. Transfer the reconstituted RNase back into the Suspension Buffer and mix thoroughly. 4. Mark the reconstituted mixture (enzyme and buffer) with the date of reconstitution and store at +2 to +8°C. ⚠ Reconstituted mixture is stable for 6 months when stored at +2 to +8°C.

	Possible Cause	Recommendation
RNA present in final product	Too many cells in starting material.	Do not overload the column.
Genomic DNA present in final product	Genomic DNA sheared during lysis step.	Do not vortex the preparation after adding Lysis Buffer.
Additional band running slightly faster than supercoiled plasmid is seen on gels	Denatured plasmid in final product.	Reduce the incubation time during step 2 (lysis step) of the protocol.

5. Additional Information on this Product

How this Product Works

The isolation procedure is based on a modified alkaline lysis protocol and can be divided into the following steps:

The bacteria are partially lysed, allowing the plasmid DNA to escape the cell wall into the supernatant. The larger *E. coli* chromosomal DNA is trapped in the cell wall. The lysate is cleared of cellular debris and the plasmid DNA containing fraction is added to the column. The bound plasmid DNA is washed to remove contaminating bacterial components. The plasmid DNA is eluted and precipitated to remove salt and to concentrate the eluate.

This is a commonly used method that generates highly purified plasmid DNA free of RNA contamination according to current quality control procedures.

Test Principle

①	Cell harvest and disruption.
②	Precipitation of chromosomal DNA.
③	Clarification of bacterial lysate.
④	Adsorption of the plasmid DNA to the matrix.
⑤	Wash to remove residual impurities.
⑥	Elute plasmid DNA with high salt buffer.
⑦	Concentration and salt removal by alcohol precipitation.

Joint values

The column matrix developed by Macherey-Nagel GmbH and Co. KG, and the proven optimal buffer composition of Roche Diagnostics, have been combined to provide you with state-of-the-art plasmid isolation product.

References

1 Birnboim, H.C. and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. *Nucl. Acids Res.* **7,** *1*513-1522
2 Sambrook, J. *et al.* (1989) Molecular Cloning: A Laboratory Manual, 2nd. Edition, Cold Spring Harbour Laboratory Press
3 Ausubel, F.M. *et al.* (eds.) (1991) Current Protocols in Molecular Biology, Wiley Interscience, New York
4 Wallerstedt, R.S. *et al.* (2004) Fast and Convenient Plasmid DNA Purification for Transient Transfection with the Genopure Kit. *Biochemica* **4**, 6-7.
5 Nemetz, C. *et al.* (2002) RTS: Rapid Protein Expression Directly from PCR Fragments *Biochemica* **2,** 22.
6 Kobayashi, N. *et al.* (2003). Vector-Based in Vivo RNA Interference: Dose- and Time-Dependent Suppression of Transgene Expression. *J of Pharmacology and Experimental Therapeutics* **308**, 688-693.
7 Song, S *et al.* (2005). Bile Acids Induce MUC2 Overexpression in Human Colon Carcinoma Cells.*Cancer* **103**,1606–1614.

Quality Control

Plasmid DNA purified using this kit has been tested for restriction digestion. pUC 19 was isolated from transformed HB101 as described in the protocol, 1 μg of plasmid was completely digested with 1 U *Msp* I for 2 hours at +37 °C as shown by agarose gel analysis.

Plasmid recovery was tested with 50 μg purified plasmid. The recovery was >90% with more than 80% in supercoiled form.

The yield of plasmid DNA was determined by isolating pBS from DH5α cells. From 30 ml culture volume with a density of A_{600} between 3 and 6 was obtained >85 μg plasmid DNA.

The purity checked by the ratio of A_{260}/A_{280} is 1.8 ± 0.2.

RNA contamination was analyzed using 3 μg pBS purified with the standard procedure, and checked by electrophoresis on an agarose gel. No RNA was detected.

Kit components have been tested for the absence of nucleases, according to current Quality Control procedures.

6. Supplementary Information

6.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this document:

Text Convention	Use
Numbered stages labeled ①, ②, *etc.*	Stages in a process that usually occur in the order listed
Numbered instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed
Asterisk *	Denotes a product available from Roche Diagnostics

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Changes to Previous Version

- Editorial changes

6.2 Ordering Information

Roche Diagnostics offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our Homepage, www.lifescience.roche.com, and our Special Interest Sites including:

www.lifescience.roche.com/napure/

	Product	Pack Size	Cat. No.
Kits	Genopure Plasmid Maxi Kit	up to 10 isolations	03 143 422 001
	Genopure Buffer Set for Low Copy Number Plasmids	1 Buffer Set	04 634 772 001
	High Pure Plasmid Isolation Kit	50 purifications 250 purifications	11 754 777 001 11 754 785 001
	High Pure PCR Template Preparation Kit	100 purifications	11 796 828 001
Single Reagents	RNAse, DNase-free	500 µg (1 ml)	11 119 915 001
	Agarose MP	100 g 500 g	11 388 983 001 11 388 991 001
	Agarose LE	100 g 500 g	11 685 660 001 11 685 678 001
	X-tremeGENE 9 DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 365 779 001 06 365 787 001 06 365 809 001
	X-tremeGENE HP DNA Transfection Reagent	0.4 ml 1 ml 5 x 1 ml	06 366 244 001 06 366 236 001 06 366 546 001
	RNase A	25 mg 100 mg	10 109 142 001 10 109 169 001

6.3 Trademarks

6.4 Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

7. Quick Reference Protocols

7.1 Quick Reference Procedure for High Copy Number Plasmids

❶
- Centrifuge bacterial cells from 5 – 30 ml culture grown in LB medium (5 – 10 min / 3,000 – 5,000 × *g* / +2 to +8°C).
- Discard the supernatant.
- Carefully resuspend the pellet in 4 ml Suspension Buffer + RNase and mix well.

❷
Add 4 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times. Incubate 2 – 3 min at +15 to +25°C.

⚠ To avoid shearing genomic DNA, do not vortex the suspension in Lysis Buffer. To prevent release of chromosomal DNA from the cell debris, do not incubate for more than 5 min.

❸
- Add 4 ml chilled Neutralization Buffer to the suspension.
- Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogenous suspension is formed.
- Incubate the tube 5 min on ice.

The solution becomes cloudy and a flocculent precipitate will form.

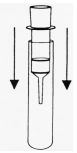
❹
Clear the lysate by either centrifugation (4a) or by filtration (4b).

4a. Centrifuge at high speed (>30 min / >12,000 × *g* / +2 to +8°C). Directly after centrifugation, carefully remove the supernatant from the white precipitate and proceed with step 5.

4b. Put a folded filter into a funnel that has been inserted into a 50 ml plastic tube.
- Moisten the filter with a few drops of Equilibration Buffer or double-distilled water.
- Load the lysate onto the wet, folded filter and collect the flow-through.

⚠ The SDS precipitates with cellular debris when Neutralization Buffer is added; this white precipitate should not be loaded onto the column since it will clog the column. If the solution obtained after step 4 is not clear, remove the remaining precipitate by passing the solution over a folded filter.

❺
- Mount the sealing ring to the column as shown in the figure to fix the column in the collection tube.
- Insert one column into one collection tube.
- Equilibrate the column with 2.5 ml Equilibration Buffer.
- Allow the column to empty by gravity flow.
- Discard the flow through.



❻
- Load the cleared lysate from step 4 onto the equilibrated column.
- Allow the column to empty by gravity flow.
- Discard the flow through.

❼
- Wash the column with 5 ml Wash Buffer.
- Allow the column to empty by gravity flow.
- Discard the flow through.

❽	• Repeat step 7. • Discard flow through and collection tube.
❾	• Re-insert the column into a new collection tube capable of withstanding high-speed centrifugation ($\geq$15,000 × *g*). • Elute the plasmid with 5 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • The collected flow-through contains the plasmid.
❿	• Precipitate the eluted plasmid DNA with 3.6 ml isopropanol (equilibrated to +15 to +25°C). • Centrifuge immediately (30 min / $\geq$15,000 × *g* / +2 to +8°C). • Carefully discard the supernatant.
⓫	• Wash the plasmid DNA with 3 ml chilled (+2 to +8°C) 70% ethanol. • Centrifuge (10 min / >15,000 × *g* / +2 to +8°C). • Carefully remove ethanol from the tube with a pipette tip. • Air-dry the plasmid DNA pellet for 10 min.
⓬	Carefully redissolve the plasmid DNA pellet in 20 - 100 µl TE buffer or sterile double-distilled water.

❶
- Centrifuge bacterial cells from 10 – 100 ml *E. coli* culture grown in LB medium (5 – 10 min / 3,000 – 5,000 × *g* / +2 to +8°C).
- Discard the supernatant.
- Carefully resuspend the pellet in 8 ml Suspension Buffer + RNase and mix well.

❷
Add 8 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times. Incubate 2 – 3 min at +15 to +25°C.

⚠ To avoid shearing genomic DNA, do not vortex the suspension in Lysis Buffer. To prevent release of chromosomal DNA from the cell debris, do not incubate for more than 5 min.

❸
- Add 8 ml chilled Neutralization Buffer to the suspension.
- Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogenous suspension is formed.
- Incubate the tube 5 min on ice.

The solution becomes cloudy and a flocculent precipitate will form.

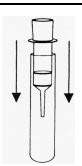
❹
Clear the lysate by either centrifugation (4a) or by filtration (4b).

4a. Centrifuge at high speed (>30 min / >12,000 × *g* / +2 to +8°C). Directly after centrifugation, carefully remove the supernatant from the white precipitate and proceed with step 5.

4b. Put a folded filter into a funnel that has been inserted into a 50 ml plastic tube.
- Moisten the filter with a few drops of Equilibration Buffer or double-distilled water.
- Load the lysate onto the wet, folded filter and collect the flow-through.

⚠ The SDS precipitates with cellular debris when Neutralization Buffer is added; this white precipitate should not be loaded onto the column since it will clog the column. If the solution obtained after step 4 is not clear, remove the remaining precipitate by passing the solution over a folded filter.

❺
- Mount the sealing ring to the column as shown in the figure to fix the column in the collection tube.
- Insert one column into one collection tube.
- Equilibrate the column with 2.5 ml Equilibration Buffer.
- Allow the column to empty by gravity flow.
- Discard the flow through.



❻
- Load the cleared lysate from step 4 onto the equilibrated column.
- Allow the column to empty by gravity flow.
- Collect the flow through and load it a second time onto the column.
- Allow the column to empty by gravity flow.
- Discard the flow through.

❼	• Wash the column with 4 ml Wash Buffer. • Allow the column to empty by gravity flow. • Discard the flow-through.
❽	Repeat step 7.
❾	• Repeat step 7. • Discard flow through and collection tube.
❿	• Re-insert the column into a new collection tube capable of withstanding high-speed centrifugation (≥15,000 × *g*). • Elute the plasmid with 2.5 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • Collect the flow through.
⓫	• Elute a second time with 2.5 ml prewarmed Elution Buffer (+50°C) and combine eluates. • Allow the column to empty by gravity flow. • The collected flow through contains the plasmid.
⓬	• Precipitate the eluted plasmid DNA with 3.6 ml isopropanol (equilibrated to +15 to +25°C). • Centrifuge immediately (30 min / ≥15,000 × *g* / +2 to +8°C). • Carefully discard the supernatant.
⓭	• Wash the plasmid DNA with 3 ml chilled (+2 to +8°C) 70% ethanol. • Centrifuge (10 min / >15,000 × *g* / +2 to +8°C). • Carefully remove ethanol from the tube with a pipette tip. • Air-dry the plasmid DNA pellet for 10 min.
⓮	Carefully redissolve the plasmid DNA pellet in 20 - 50 µl TE buffer or sterile double-distilled water.

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