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Genopure Buffer Set for Low Copy Number Plasmids

Version 05

Content version: April 2016

Supplementary set to be used only in combination with the Genopure Plasmid Kits

Cat. No. 04 634 772 001 1 Buffer Set

Store the kit at +15 to +25°C

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Table of Contents

PROTOCOL

1. What this Product Does

Number of Tests

The Buffer Set is a supplement to the Genopure Plasmid Kits to improve the alkaline lysis. The set provides enough additional buffer for:

- 20 Maxi preps with the Genopure Plasmid Maxi Kit (each for isolation of up to 500 µg plasmid DNA from 100 to 500 ml bacterial culture), or
- 60 Midi preps with the Genopure Plasmid Midi Kit (each for isolation of up to 100 µg plasmid DNA from 10 to 100 ml bacterial culture).

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 with yellow label	Suspension Buffer	• 4× 125 ml • for suspension of bacterial cell pellets
2 blue	RNase A	• 4× 12 mg • to be dissolved in Suspension Buffer
3 green	Lysis Buffer	• 4× 125 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	• 4× 125 ml • to form a stable cellular debris precipitate

Storage and Stability

Store the set at +15 to +25°C, where it will be stable until the expiration date printed on the label. The set 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.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform reactions include:

- Genopure Plasmid Maxi Kit*
- Genopure Plasmid Midi Kit*

Application

This set provides extra buffer that may be used with either of the Genopure Plasmid Kits. Doubling the volume of the Suspension, Lysis and Neutralization buffers will help ensure an efficient yield of low copy number plasmids from bacterial culture. The increased buffer volume is required for efficient alkaline lysis.

Assay Time

Total time	60 to 75 min including a filtration step after alkaline lysis
Hands-on time	Minimal hands-on time required (about 10 minutes)

2. How To Use this Product

2.1 Before You Begin

Precautions

Lysis buffer (bottle 3) contains Sodium hydroxide. Irritating to eyes and skin. Keep away from food, drink and animal feeding stuffs. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protecting clothing. If swallowed, seek medical advice immediately and show this container or label.

Sample Material

For Midi preps: 10 – 100 ml *E. coli* culture, transformed with a low copy number plasmid

For Maxi preps: 100 – 500 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.

Preparation of Working Solutions

Ⓢ The Lysis Buffer and the Neutralization Buffer supplied in the set are ready to use.

Prepare the following working solution before using the buffer set:

Suspension Buffer/RNase A

To dissolve the lyophilized enzyme in Suspension Buffer, pipet 1 ml of Suspension Buffer (bottle 1, colorless cap with yellow label) 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 enough working solution for 60 Midi preps (isolation of up to 100 µg plasmid DNA/preparation) or 20 Maxi preps (isolation of up to 500 µg plasmid DNA preparation). Alternatively,

Ⓢ If preparing aliquots of the working solution, remember that the final concentration of RNase A in the working solution must be 100 µg/ml.

Reconstituted buffer is stable for 6 months if stored properly (+2 to +8°C).

Depending on the copy number of the plasmids, start with 10 to 100 ml (Midi kit) or 100 to 500 ml (Maxi kit) bacterial suspension for each preparation.

For details of the plasmid isolation procedure, see section 3 in the the Instructions for Use of the Genopure Plasmid Midi / Maxi Kit. To prepare up to 100 µg plasmid DNA, use the Midi prep procedure. To prepare up to 500 µg plasmid DNA, use the Maxi prep procedure. Modify each procedure by doubling the amounts of Suspension, Lysis and Neutralization Buffers.

See the Quick Reference Protocols in section 7 of this document. Note the increased amounts of buffers required for isolation of low copy plasmid DNA.

3. Results

For typical results, see the Instructions of Use of the Genopure Plasmid Midi / Maxi Kit.

	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 any stuck to 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 properly.

	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 of the kit is based on a modified alkaline lysis protocol. The bacteria are partially lysed, allowing the plasmid DNA to escape into the supernatant. The larger *E. coli* chromosomal DNA is trapped in the cell wall. Cellular debris is cleared from the lysate and the supernatant containing plasmid DNA is added to a 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 commonly used method generates highly purified plasmid DNA free of RNA contamination according to current quality control procedures.

The quality of the plasmid DNA obtained with this procedure is better than that of plasmid DNA obtained after 2 × CsCl gradient centrifugation. Plasmid DNA isolated with the Genopure Plasmid Midi / Maxi Kit is suitable for all molecular biology applications, *e.g.,* transfection, PCR, restriction analysis, Southern blotting, sequencing and cloning.

Test Principle

The isolation method is optimized for cultures grown in LB media; other rich media may require increased volumes of Suspension, Lysis and Neutralization Buffer, plus an additional wash step. The isolation procedure is suitable for all sizes of plasmid; however, lysates containing larger constructs (up to 100 kb) should be cleared by filtration (rather than centrifugation) so the plasmids are not sheared.

The yield of plasmid DNA depends on several parameters, *e.g.,* quality of the bacterial culture growth, amount of culture suspension used for the preparation, kind of plasmid, *etc.* As a rule of thumb, the typical yield of low copy number plasmids is about 0.2 to 1 µg of DNA per ml of original bacterial culture.

References

1 Birnboim, H.C. and Doly, J. (1979) *Nucl. Acids Res.* **7**, 1513-1522.
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) *Biochemica* **4**, 6-7.
5 Nemetz, C. *et al.* (2002) *Biochemica* **2,** 22.

Quality Control

Plasmid DNA purified by this kit has been tested for restriction digestion. pUC 19 was isolated from transformed HB101, as described in the protocol of the Instructions for Use of the Genopure Plasmid Midi / Maxi Kit. One unit *Msp* I could completely digest 1 µg of plasmid in 2 hours at +37°C, as shown by agarose gel analysis.

Plasmid recovery was tested with 250 µg purified plasmid. The recovery was >90%, with more than 80% in supercoiled form.

The yield and purity of plasmid DNA was determined by isolating pBS from DH5α cells. From 150 ml culture (density at harvest, $A_{600} = 3$ to 6), the protocol obtained >400 µg plasmid DNA. The ratio of A_{260}/A_{280} was 1.8 ± 0.2, indicating the purity of the preparation. When 3 µl of the purified pBS preparation was examined electrophoretically on an agarose gel, no RNA contamination was detected.

The set components have been tested for the absence of nucleases.

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

- Changes of screw cap color

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 home page 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 Plasmid Midi Kit	up to 20 isolations	03 143 414 001
	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

HIGH PURE, GENOPURE, and X-TREMEGENE are trademarks of Roche.

All other product names and trademarks are the property of their respective owners.

6.4 Regulatory Disclaimer

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

7. Quick Reference Protocols for Low Copy Number Plasmids

7.1 Quick Reference Protocol for Genopure Plasmid Midi Kit

⚠ SDS in the Lysis Buffer may crystallize at temperatures <+20°C. Make sure all solutions are free of crystals before use. If crystals have formed, warm solution to +37°C to dissolve them. Mix solution well before use.

Please note that the protocol is adapted to the need of low copy number preparations.

❶
- Grow *E.coli* in LB medium. Harvest bacterial cells from 10 – 100 ml culture by centrifuging (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.

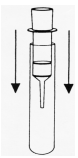
❹ Clear the lysate by either centrifugation (4a) or by filtration (4b).

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 the 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 figure 1 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.



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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 4 ml Wash Buffer. • Allow the column to empty by gravity flow. • Discard the flow-through.
❽	Repeat step 7.
❾	Repeat step 7.
❿	• Discard flow-through and collection tube. • 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. • The collected flow-through contains the plasmid.
⓫	• Elute a second time with 2.5 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • Combine both flow-throughs.
⓬	• Precipitate 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.

In these steps, the volume has been doubled. Use buffer from the buffer set to supplement the buffer supplied in the plasmid kit.

⚠ SDS in the Lysis Buffer may crystallize at temperatures <+20°C. Make sure all solutions are free of crystals before use. If crystals have formed, warm solution to +37°C to dissolve them. Mix solution well before use.

Please note that the protocol is adapted to the need of low copy number preparations.

1
- Grow *E.coli* in LB medium. Harvest bacterial cells from 100 – 500 ml culture by centrifuging (5 – 10 min / 3,000 – 5,000 × *g* / +2 to +8°C).
- Discard the supernatant.
- Carefully resuspend the pellet in 24 ml# Suspension Buffer + RNase and mix well.

2
Add 24 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.

3
- Add 24 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.

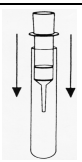
4
Clear the lysate by either centrifugation (4a) or by filtration (4b).

4a Centrifuge at high speed (>45 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 sterile 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.

5
- Mount the sealing ring to the column as shown in figure 1 to fix the column in the collection tube.
- Insert one column into one collection tube.
- Equilibrate the column with 6 ml Equilibration Buffer.
- Allow the column to empty by gravity flow.
- Discard the flow-through.



6
- Load the cleared lysate from step 4 onto the equilibrated column.
- Allow the column to empty by gravity flow.
- Discard the flow-through.

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❼	• Wash the column with 12 ml Wash Buffer. • Allow the column to empty by gravity flow. • Discard the flow-through.
❽	Repeat step 7.
❾	Repeat step 7.
❿	• Discard flow-through and collection tube. • Re-insert the column into a new collection tube capable of withstanding high-speed centrifugation ($\geqslant$15,000 × *g*). • Elute the plasmid with 7 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • The collected flow-through contains the plasmid.
⓫	• Elute a second time with 7 ml prewarmed Elution Buffer (+50°C). • Allow the column to empty by gravity flow. • Combine both flow-throughs.
⓬	• Precipitate the eluted plasmid DNA with 10 ml isopropanol (equilibrated to +15 to +25°C). • Centrifuge immediately (30 min / $\geqslant$15,000 × *g* / +2 to +8°C). • Carefully discard the supernatant.
⓭	• Wash the plasmid DNA with 4 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 100 - 500 µl TE buffer or sterile double-distilled water.

In these steps, the volume has been doubled. Use buffer from the buffer set to supplement the buffer supplied in the plasmid kit.

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