

## Genopure Plasmid Midi Kit

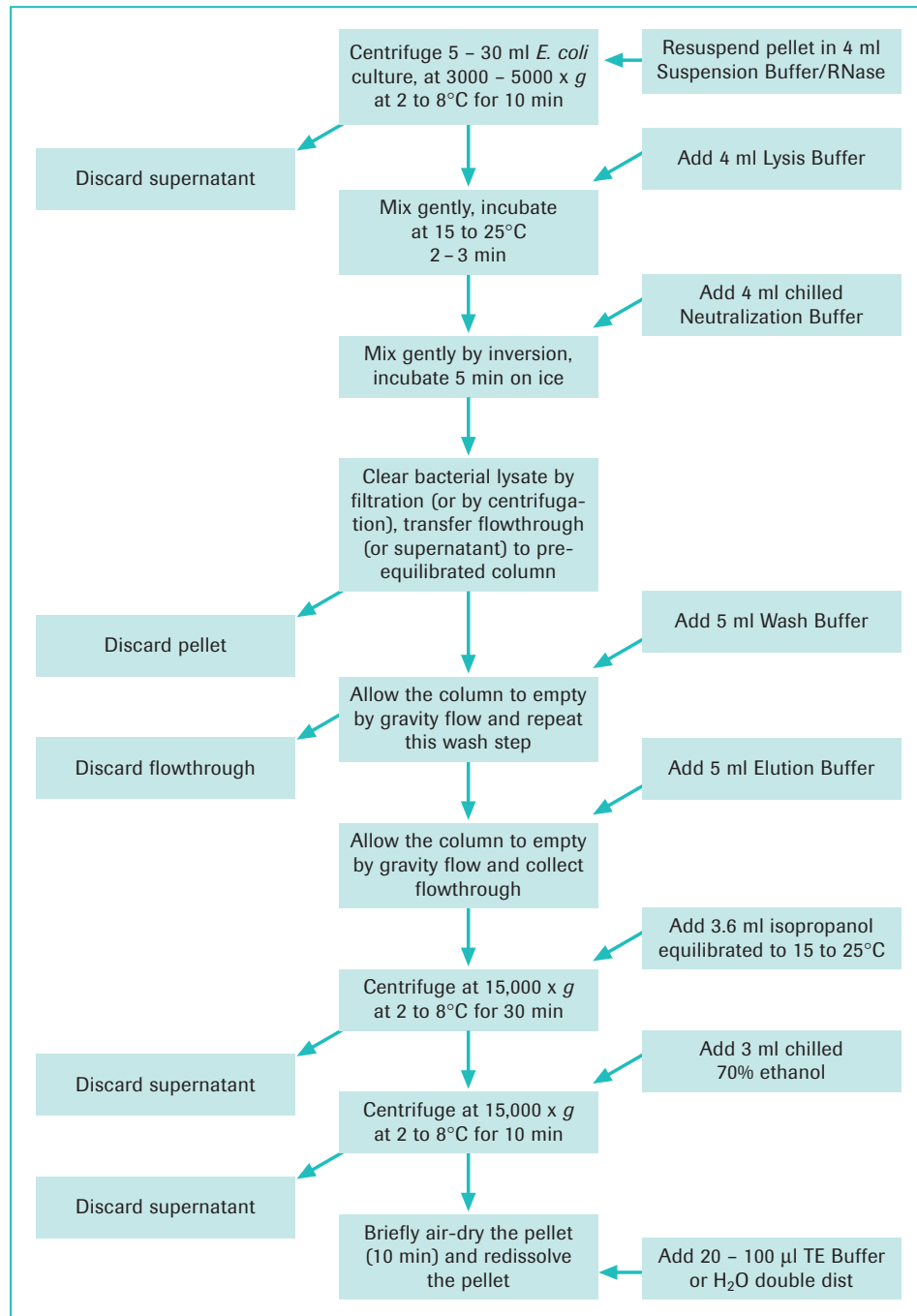
for up to 20 preparations of plasmid DNA in medium scale

Cat. No. 03 143 414 001

<b>Principle</b>	<p>The isolation procedure is based on a modified alkaline lysis protocol and can be divided into the following steps:</p> <p>The bacteria are partially lysed, allowing the plasmid DNA to escape the cell wall into the supernatant. The larger <i>E. coli</i> 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.</p> <p>This is a commonly used method that generates highly purified plasmid DNA (free of RNA contamination).</p>
<b>Starting material</b>	<ul style="list-style-type: none"> <li>● 5 – 30 ml <i>E. coli</i> cultures transformed with a high copy number plasmid.</li> <li>● 10 – 100 ml <i>E. coli</i> cultures transformed with a low copy number plasmid (at a density of 2 – 6.0 A<sub>600</sub> units per ml bacterial culture).</li> </ul>
<b>Application</b>	<p>This kit is used to prepare plasmid DNA in medium quantities known as "midi preps". Using a modified alkaline lysis method highly purified plasmid DNA is generated. The kit is designed for the isolation of up to 100 µg of plasmid DNA from bacterial culture.</p> <p>Depending on the copy number of the plasmids use either 5 to 30 ml (high copy number) or 10 to 100 ml (low copy number) bacterial suspension. The quality of the plasmid DNA is better than plasmid DNA obtained by 2 x CsCl gradient centrifugation. As a result, the plasmid DNA is suitable for all molecular biology applications <i>e.g.</i>, transfection, PCR, restriction analysis/Southern blotting, sequencing and cloning.</p>
<b>Time required</b>	<ul style="list-style-type: none"> <li>● Total time: 60 min including a filtration step after the alkaline lysis.</li> <li>● Hands-on time: Minimal hands-on time required (about 10 min).</li> </ul>
<b>Results</b>	<ul style="list-style-type: none"> <li>● Purity: Plasmid DNA is free of all other bacterial components, including RNA, shown by gel electrophoresis</li> <li>● Yield: Depending on <i>E. coli</i> strain and density of the cell culture. Comparable to traditional purification methods</li> <li>● Application: The purified plasmid has been used for PCR, sequencing and transfection with excellent results</li> </ul>
<b>Benefits</b>	<ul style="list-style-type: none"> <li>● <b>Avoids organic or toxic materials</b> as no phenol or chloroform, CsCl and ethidium bromide needed</li> <li>● <b>Ready to use</b> because all reagents provided with the kit</li> <li>● <b>Reliable quality</b> because better than 2 x CsCl</li> <li>● <b>Parallel processing</b> because of use of high speed gravity flow columns</li> <li>● <b>All plasmid sizes</b> can be isolated even BAC DNA</li> </ul>

## How to use the kit

### I. Flow diagram



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## II. Kit contents

- Suspension Buffer (100 ml) for suspension of bacterial cell pellets
- RNase A (12 mg) for dissolution in Suspension Buffer
- Lysis Buffer (100 ml) for bacterial cell lysis
- Neutralization Buffer (100 ml) to form a stable cellular debris precipitate
- Equilibration Buffer (70 ml) for equilibrating the columns prior to use
- Wash Buffer (250 ml) for removal of residual impurities
- Elution Buffer (125 ml) for plasmid elution
- NucleoBond AX 100 Columns (20 columns) for the isolation step
- Folded filters (20 filters) to eliminate a centrifugation step and to remove cellular debris
- Sealing rings (10 rings) to station the columns in test tubes

## III. Additional materials needed

- Centrifuge and tubes for harvesting bacterial cultures, capable of  $\geq 15,000 \times g$
- 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

## IV. Protocol for preparing high copy number plasmid DNA

Step	Action	Time / x g / Temperature
1	<ul style="list-style-type: none"> <li>▶ Centrifuge bacterial cells from 5 – 30 ml <i>E. coli</i> culture grown in LB medium.</li> <li>▶ Discard the supernatant.</li> <li>▶ Carefully resuspend the pellet in 4 ml Suspension Buffer + RNase and mix well.</li> </ul>	5 – 10 min/ 3000 – 5000 x g/ 2 to 8°C
2	<ul style="list-style-type: none"> <li>▶ Add 4 ml Lysis Buffer to the suspension and mix gently by inverting the tube 6 to 8 times and incubate.</li> </ul> <p><b>!</b> <i>Do not vortex in order to avoid shearing and release of genomic DNA. Do not incubate for more than 5 min to prevent the release of chromosomal DNA from the cell debris.</i></p>	2 – 3 min at 15 to 25°C
3	<ul style="list-style-type: none"> <li>▶ Add 4 ml chilled Neutralization Buffer to the suspension.</li> <li>▶ Immediately mix the suspension gently by inverting the tube 6 to 8 times until a homogeneous suspension is formed.</li> <li>▶ Incubate the tube.</li> </ul> <p><b>!</b> <i>The solution becomes cloudy and flocculent precipitate will form.</i></p>	5 min on ice
4	<ul style="list-style-type: none"> <li>▶ Clear the lysate by either centrifugation (4a) or by filtration (4b).</li> </ul>	
4a	<ul style="list-style-type: none"> <li>▶ Centrifuge at high speed</li> <li>▶ Directly after centrifugation carefully remove the supernatant from the white precipitate and proceed with step 5.</li> </ul>	>30 min/ >12,000 x g/ 2 to 8°C
4b	<ul style="list-style-type: none"> <li>▶ Put a folded filter into a funnel inserted in a 50 ml plastic tube.</li> <li>▶ Moisten the filter with a few drops of Equilibration Buffer or sterile double dist. water.</li> <li>▶ Load the lysate onto the wet folded filter and collect the flowthrough.</li> </ul> <p><b>!</b> <i>The SDS is removed with the Neutralization Buffer (white precipitate) and should not be loaded onto the column. If the supernatant is not clear, load it again onto a folded filter to prevent clogging of the column.</i></p>	
5	<ul style="list-style-type: none"> <li>▶ Mount the sealing ring to the column as shown in Figure 36 to fix the column in the Collection Tube.</li> <li>▶ Insert one column into one Collection Tube.</li> <li>▶ Equilibrate the column with 2.5 ml Equilibration Buffer.</li> <li>▶ Allow the column to empty by gravity flow.</li> <li>▶ Discard the flowthrough.</li> </ul>	Figure 36 
6	<ul style="list-style-type: none"> <li>▶ Load the cleared lysate of step 4 onto the equilibrated column.</li> <li>▶ Allow the column to empty by gravity flow.</li> <li>▶ Discard the flowthrough.</li> </ul>	
7	<ul style="list-style-type: none"> <li>▶ Wash the column with 5 ml Wash Buffer.</li> <li>▶ Allow the column to empty by gravity flow.</li> <li>▶ Discard the flowthrough.</li> </ul>	
8	<ul style="list-style-type: none"> <li>▶ Repeat step 7.</li> <li>▶ Discard flowthrough and Collection Tube.</li> </ul>	



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## IV. Protocol for preparing high copy number plasmid DNA,

continued

Step	Action	Time / x g / Temperature
9	<ul style="list-style-type: none"> <li>▶ Re-insert the column into a new Collection Tube capable of withstanding high speed centrifugation (<math>\geq 15,000 \times g</math>).</li> <li>▶ Elute the plasmid with 5 ml Elution Buffer.</li> <li>▶ Allow the column to empty by gravity flow.</li> <li>▶ The collected flowthrough contains the plasmid.</li> </ul>	
10	<ul style="list-style-type: none"> <li>▶ Precipitate the eluted plasmid DNA with 3.6 ml isopropanol equilibrated to 15 to 25°C.</li> <li>▶ Centrifuge immediately at high speed.</li> <li>▶ Carefully discard the supernatant.</li> </ul>	30 min/ $\geq 15,000 \times g/2$ to 8°C
11	<ul style="list-style-type: none"> <li>▶ Wash the plasmid DNA with 3 ml chilled 70% ethanol.</li> <li>▶ Centrifuge at high speed.</li> <li>▶ Carefully remove ethanol from the tube with pipet tip.</li> <li>▶ Air-dry the plasmid DNA pellet.</li> </ul>	2 to 8°C 10 min/ $>15,000 \times g/2$ to 8°C 10 min
12	<ul style="list-style-type: none"> <li>▶ Carefully redissolve the plasmid DNA pellet in 20 – 100 <math>\mu</math>l TE Buffer or sterile double dist. H<sub>2</sub>O.</li> </ul>	

## V. Troubleshooting the Genopure Plasmid Midi protocol

If you get...	Then, the cause may be...	And you should...
Low nucleic acid yield or purity	Kit stored under non-optimal conditions	▶ Store kit at 15 to 25°C at all times upon arrival.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness	<ul style="list-style-type: none"> <li>▶ Store all buffers at 15 to 25°C.</li> <li>▶ After reconstitution of RNase with Suspension Buffer store aliquots at 2 to 8°C.</li> <li>▶ Close all reagent bottles tightly after each use to preserve pH, stability and freedom from contamination.</li> </ul>
	Reagents and samples not completely mixed	<ul style="list-style-type: none"> <li>▶ Always mix the sample tube well after addition of each reagent.</li> <li>▶ Ensure Lysis Buffer and Neutralization Buffer are free of precipitates.</li> </ul>
Low recovery of nucleic acids after elution	Non-optimal reagent has been used for elution. Salt is required for optimal elution	▶ Use the Elution Buffer of the kit.

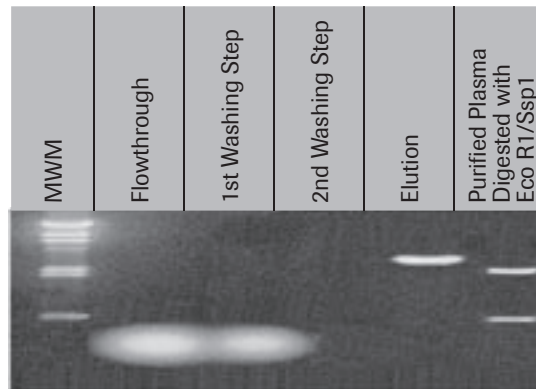


## V. Troubleshooting the Genopure Plasmid Midi protocol, continued

If you get...	Then, the cause may be...	And you should...
Low plasmid yield	Too few cells in starting material	▶ Grow <i>E. coli</i> to an absorbency ( $A_{600}$ ) of 2 – 6 before harvest.
	Incomplete cell lysis	▶ Ensure the <i>E. coli</i> pellet is completely resuspended in Suspension Buffer. ▶ Make sure the lysate is clear and viscous after the lysis step (incubation with Lysis Buffer). ▶ Ensure a cloudy white precipitate forms when Binding 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 is present in final product	RNase not completely dissolved	▶ To reconstitute the lyophilized RNase completely: 1. Pipette 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 all 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.  ! <i>Reconstituted mixture is stable for 6 months when stored properly.</i>
Genomic DNA present in final product	Genomic DNA sheared during lysis step	▶ Vortexing the preparation after addition of Lysis Buffer should be avoided.
RNase present in final product	RNase not completely dissolved	▶ See suggestions under "RNA present in final product" above.
	Too many cells in starting material	▶ Do not overload the column.
Additional band running slightly faster than super-coiled plasmid is seen on gels	Denatured plasmid in final product	▶ Reduce the incubation time during step 2 (lysis step) of the protocol.

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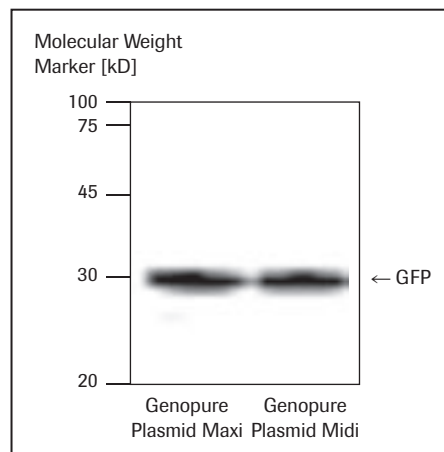
## Typical results with the kit



**Figure 37: The purification process results in contamination-free plasmid DNA.**

Plasmid Preparation Method	Endotoxin (EU/ $\mu$ g)	Transfection efficiency (%)
Genopure	4 – 10	100
2-fold CsCl	0.7 – 3	~95
Alternative Commercial Source (Anion Exchange)	9.3	$\geq$ 80
Silica-Matrix/Gel Slurry	> 1000	> 30

**Table 2: Mean values from several experiments.**



**Figure 38: Western blot analysis of GFP after expression in RTS 500 HY reactions.** Equal volumes of diluted reaction solutions were separated by SDS-PAGE, then blotted. Detection was performed with the anti-His<sub>6</sub> peroxidase-conjugated antibody.

## References

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