

## High Pure Plasmid Isolation Kit

for purification of plasmid DNA from bacterial cultures

Cat. No. 11 754 777 001 (up to 50 purifications)

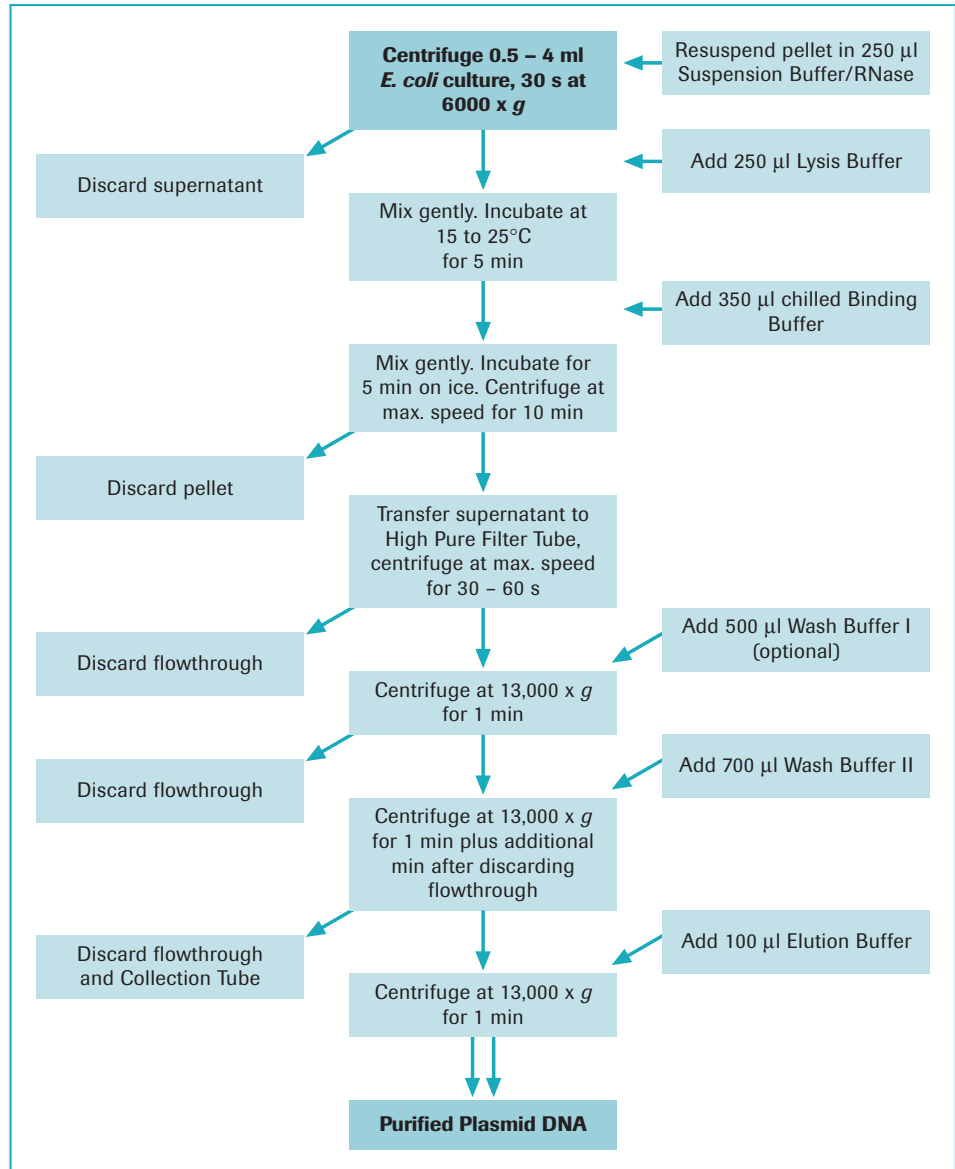
Cat. No. 11 754 785 001 (up to 250 purifications)

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<b>Principle</b>	Alkaline lysis releases plasmid DNA from bacteria and RNase removes all RNA in the lysate. Then, in the presence of a chaotropic salt (guanidine HCl), plasmid DNA binds selectively to glass fiber fleece in a special centrifuge tube. The DNA remains bound while a series of rapid “wash-and-spin” steps remove contaminating bacterial components. Finally, low salt elution removes the DNA from the glass fiber fleece. The process does not require DNA precipitation, organic solvent extractions, or extensive handling of the DNA.
<b>Starting material</b>	<ul style="list-style-type: none"> <li>● 0.5 – 4.0 ml recombinant <i>E. coli</i> cultures research samples (at a density of 1.5 – 5.0 A<sub>600</sub> units per ml)</li> </ul>
<b>Application</b>	<ul style="list-style-type: none"> <li>● Preparation of up to 15 µg purified plasmid DNA from bacterial cultures, which may be used directly for PCR, cloning, sequencing, <i>in vitro</i> transcription, or generation of labeled hybridization probes</li> </ul>
<b>Time required</b>	<ul style="list-style-type: none"> <li>● Total time: approx. 30 min</li> <li>● Hands-on time: Minimal hands-on time required</li> </ul>
<b>Results</b>	<ul style="list-style-type: none"> <li>● Yield: Variable, depending on the <i>E. coli</i> strain (See the table under Part IV of “How to use the kit” in this article)</li> <li>● Purity: Plasmid DNA is free of all other bacterial components, including RNA</li> </ul>
<b>Benefits</b>	<ul style="list-style-type: none"> <li>● <b>Saves time</b>, because the kit can prepare up to 24 plasmid samples in less than 30 min</li> <li>● <b>Minimizes DNA loss</b>, because the kit removes contaminants without precipitation or other handling steps that can lead to lost or degraded DNA</li> <li>● <b>Increases lab safety</b>, because the kit does not use hazardous organic reagents such as cesium chloride, phenol, chloroform, or ethidium bromide</li> <li>● <b>Improves reliability and reproducibility of downstream procedures</b>, because the kit removes RNA and other impurities that might cause the plasmid DNA to behave unpredictably</li> </ul>

## How to use the kit

### I. Flow diagram



### II. Kit contents

- Suspension Buffer (25 or 80 ml)
- RNase A powder (2.5 mg or 8 mg)
  - ⚠ *Dissolve RNase powder in Suspension Buffer before use.*
- Lysis Buffer (25 or 80 ml)
- Binding Buffer with guanidine HCl (25 or 100 ml)

Wash Buffer I containing guanidine HCl (33 or 100 ml); (optional; please see step 7 of protocol)

- ⚠ *Add absolute ethanol (20 ml or 60 ml) to Wash Buffer before use*

- Wash Buffer II (10 ml or 50 ml)
  - ! Add absolute ethanol (40 ml or 200 ml) to Wash Buffer before use
- Elution Buffer (Tris-HCl Buffer, pH 8.5) (40 ml)
- High Pure Filter Tubes (50 or 250 tubes)
- Collection Tubes, 2 ml (50 or 250 tubes)
  - ! Both sizes of the kit contain the same components; only the amount (values in parentheses) of the components in the kit changes.

### III. Additional materials needed

- Absolute ethanol
- Centrifuge tubes and centrifuge for harvesting up to 4 ml bacterial culture
- Standard tabletop microcentrifuge capable of a 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

### IV. Typical DNA recovery from various *E. coli* strains/pUC 19

<i>E. coli</i> host strain/density	Culture volume			
	0.5 ml	1.0 ml	2.0 ml	4.0 ml
XL 1 blue (3.6 $A_{600}$ /ml)	4.9 $\mu$ g	8.6 $\mu$ g	11.8 $\mu$ g	14.6 $\mu$ g
DH 5 $\alpha$ (1.5 $A_{600}$ /ml)	0.9 $\mu$ g	1.7 $\mu$ g	3.3 $\mu$ g	6.2 $\mu$ g
HB 101 (4.7 $A_{600}$ /ml)	1.8 $\mu$ g	3.5 $\mu$ g	5.9 $\mu$ g	8.2 $\mu$ g

### V. Protocol for preparing DNA from 0.5 – 4.0 ml of *E. coli* cell culture

- 1 Place Binding Buffer on ice.
- 2 Prepare the starting material:
  - ▶ Pellet the bacterial cells from 0.5 – 4.0 ml of *E. coli* culture.
    - ! The cells should have a density of 1.5 – 5.0  $A_{600}$  units per ml.
  - ▶ Discard the supernatant.
  - ▶ Add 250  $\mu$ l Suspension Buffer + RNase to the centrifuge tube containing the bacterial pellet.
  - ▶ Resuspend the bacterial pellet and mix well.
- 3 Treat the resuspended bacterial pellet as follows:
  - ▶ Add 250  $\mu$ l Lysis Buffer.
  - ▶ Mix gently by inverting the tube 3 to 6 times.
    - ! To avoid shearing genomic DNA, do not vortex!
  - ▶ Incubate for 5 min at 15 to 25°C.
    - ! Do not incubate for more than 5 min!



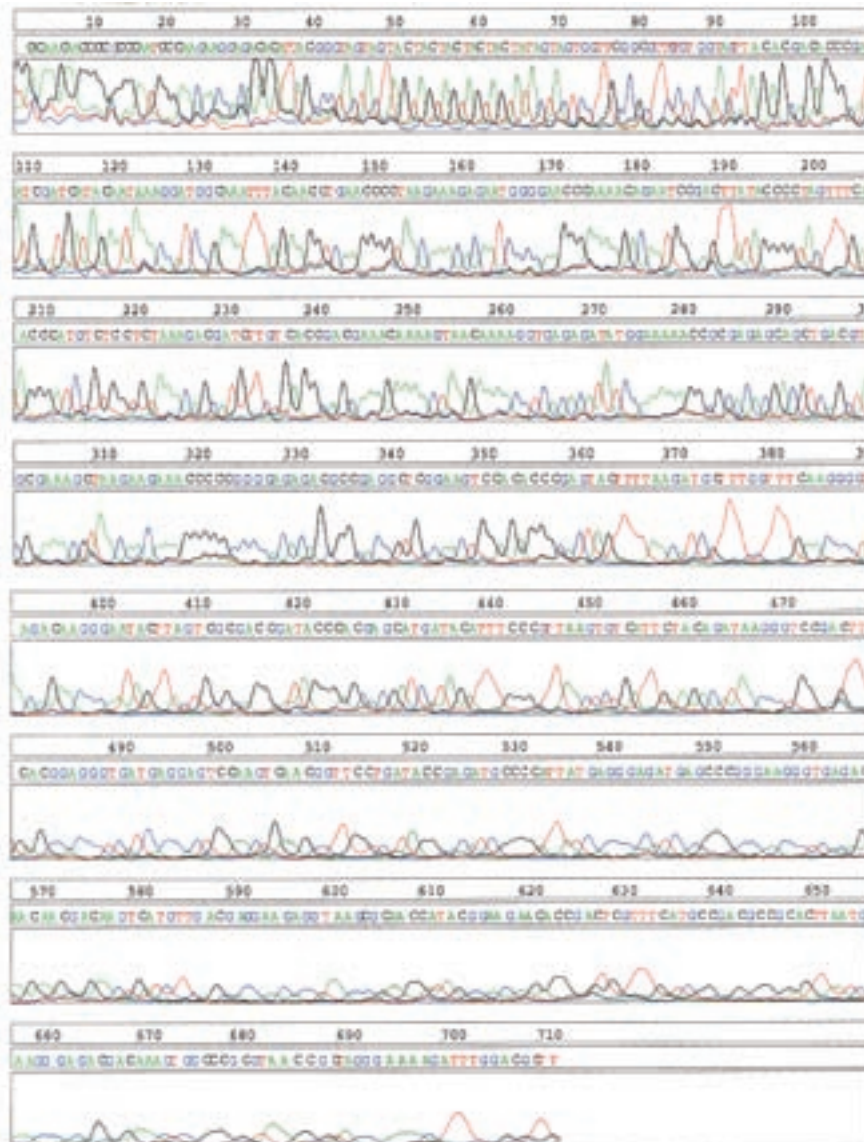
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- 4 Treat the lysed solution as follows:
- ▶ Add 350  $\mu$ l chilled Binding Buffer.
  - ▶ Mix gently by inverting the tube 3 to 6 times.
  - ▶ Incubate on ice for 5 min.
- ! *The solution should become cloudy and a flocculant precipitate should form.*
- 5 Centrifuge for 10 min at approx. 13,000  $\times$  *g* (full speed) in a standard tabletop microcentrifuge.
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- 6 After centrifugation:
- ▶ Insert one High Pure Filter Tube into one Collection Tube.
  - ▶ Transfer entire supernatant from Step 5 into upper buffer reservoir of the Filter Tube.
  - ▶ Insert the entire High Pure Tube assembly into a standard tabletop microcentrifuge.
  - ▶ Centrifuge for 30 – 60 s at full speed.
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- 7 After centrifugation:
- ▶ Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and reinsert the Filter Tube in the same Collection Tube.
  - ▶ Does the *E. coli* strain have high nuclease activity?
  - ▶ If no, go to Step 8.
- Example:** *XL 1 blue and DH 5  $\alpha$  do not have high nuclease activity and do not require the optional wash with Wash Buffer I.*
- ▶ If yes, continue this step with the optional wash.
- Example:** *HB 101 and JM strains have high nuclease activity and require the optional wash with Wash Buffer I.*
- ▶ (Optional) To eliminate high nuclease activity from the preparation:
  - ▶ Add 500  $\mu$ l of Wash Buffer I to the upper reservoir of the Filter Tube.
  - ▶ Centrifuge for 30 – 60 s at full speed and discard the flowthrough.
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- 8 To wash the cells:
- ▶ Add 700  $\mu$ l Wash Buffer II to the upper reservoir of the Filter Tube.
  - ▶ Centrifuge for 30 – 60 s at full speed and discard the flowthrough.
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- 9 After discarding the flowthrough liquid:
- ▶ Centrifuge the entire High Pure Tube assembly for an additional 30 to 60 s to remove residual Wash Buffer.
  - ▶ Discard the Collection Tube.
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- 10 To elute the DNA:
- ▶ Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
  - ▶ Add 100  $\mu$ l Elution Buffer or double dist. water (pH adjusted to 8.0 – 8.5) to the upper reservoir of the Filter Tube.
  - ▶ Centrifuge the tube assembly for 30 – 60 s at full speed.
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- 11 The microcentrifuge tube now contains the eluted plasmid DNA. You may:
- ▶ EITHER use the eluted DNA directly in such applications as cloning or sequencing
  - ▶ OR store the eluted DNA at 2 to 8°C or –15 to –25°C for later analysis.
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## VI. Troubleshooting the High Pure protocols

The same troubleshooting procedure can be applied to all High Pure kits. For details on how to troubleshoot the above protocol, see the General Troubleshooting Procedure for all High Pure kits on page 98 of this manual. For factors that may affect the High Pure Plasmid Isolation Kit, see page 101.

### Typical results with the kit



**Figure 11: Automated sequencing of plasmid DNA purified with the High Pure Plasmid Isolation Kit.** A derivative of pUC18 containing a 3.3 kb insert was isolated from *E. coli* XL 1 blue (1.5 ml culture) according to the protocol given in this article. Isolated plasmid DNA (250 ng) was sequenced with fluorescent-labeled sequencing primer under the following cycle sequencing conditions: initial denaturation, 2 min at 95°C; then, repeated cycles of denaturation (30 s, 95°C), primer annealing (30 s, 60°C), and extension/termination (60 s, 70°C). Aliquots (2 µl) of the sequencing mixture were applied to a 4.3% PAA gel (66 cm plate). The sequence was analyzed with a LI-COR Automated DNA Sequencer (Model 4000 S) in the autosequencing mode.

**Result:** More than 700 nucleotides can be read with High Pure Plasmid Isolation Kit prepared sequencing template on a LI-COR model 4000 S automated sequencing system using automated base calling and the autostop function.

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## References

- Binnicker, M. J. et al. (2007) *J. Clin. Microbiol.*, **45**, 173 – 178
- Den Hengst, C. D. (2006) *J. Bacteriol.*, **188**, 3280 – 3289
- Eilenberg, H. et al. (2006) *J. Exp. Bot.*, **57**, 2775 – 2784
- Fernández, A. et al. (2007) *J. Animicrob. Chemother.*, **59**, 841 – 847
- Grinberg, I. et al. (2006) *J. Bacteriol.*, **188**, 7635 – 7644
- Musovic, S. et al. (2006) *Appl. Envir. Microbiol.*, **72**, 6687 – 6692
- Nogales, J. et al. (2007) *Microbiology*, **153**, 357 – 365
- Steinbrenner, J. et al. (2006) *Appl. Envir. Microbiol.*, **72**, 7477 – 7484
- Susanna, K. A. et al. (2007) *J. Bacteriol.*, **189**, 4718 – 4728
- White, D. et al. (2006) *J. Biol. Chem.*, **281**, 32375 – 32384

