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High Pure Plasmid Isolation Kit

Version 10

Content version: August 2014

For small-scale (mini) preparations of purified plasmid DNA

Cat. No. 11 754 777 001 Kit for 50 purifications

Cat. No. 11 754 785 001 Kit for 250 purifications

Store the kit at +15 to +25°C.

⚠ If properly stored, all kit components are stable until the expiration date printed on the label.

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PROTOCOL

PROTOCOL

1. What this Product Does

Number of Tests

The High Pure Plasmid Isolation Kit is available in two pack sizes:

Cat. Nos.	Pack size
11 754 777 001	50 purifications
11 754 785 001	250 purifications

Both sizes of the kit contain the same components; only the amount (values in parentheses below) of the components in the kit changes.

Kit Contents

⚠ All solutions are clear. If any solution contains a precipitate, do not use it. Instead, warm the solution at room temperature or in a +37°C water bath to dissolve the precipitate.

Vial/Cap	Label	Contents / Function
1 white cap	Suspension Buffer	• 25 ml (80 ml) • 50 mM Tris-HCl and 10 mM EDTA, pH 8.0 (+25° C)
1a white cap	RNase A	• 2.5 mg (8 mg) • dry powder to be dissolved in Suspension Buffer
2 red cap	Lysis Buffer	• 25 ml (80 ml) • 0.2 M NaOH and 1% SDS
3 green cap	Binding Buffer	• 25 ml (100 ml) • 4 M guanidine hydrochloride and 0.5 M potassium acetate, pH 4.2
4 black cap	Wash Buffer I	• 33 ml (100 ml); add 20 ml (60 ml) absolute ethanol • 5 M guanidine hydrochloride, 20 mM Tris-HCl, pH 6.6 (+25°C) (final concentrations after addition of ethanol) • for performing an optional wash step of strains with high nuclease activity (*e.g., E. coli* HB101).
5 blue cap	Wash Buffer II	• 10 ml (50 ml); add 40 ml (200 ml) absolute ethanol • 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) (final concentrations after addition of ethanol) • for strains with low nuclease activity

Vial/Cap	Label	Contents / Function
6 colorless cap	Elution Buffer	• 40 ml • 10 mM Tris-HCl, pH 8.5 (+25°C)
7	High Pure Filter Tubes	One bag (5 bags) containing 50 polypropylene tubes with two layers of glass fiber fleece, for processing up to 700 µl sample volume.
8	Collection Tubes	One bag (5 bags) containing 50 polypropylene tubes (2 ml).

Storage and Stability

The High Pure Plasmid Isolation Kit components should be stored at room temperature (+15 to +25°C). Kit components are guaranteed to be stable until the expiration date printed on the label.

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

⚠ Improper storage of the kit at +2 to +8°C (refrigerator) or -15 to -25°C (freezer) may lead to formation of salt precipitates in the buffers which will adversely affect plasmid DNA purification. Therefore, High Pure isolation kits are always shipped at ambient temperature.

Additional Equipment and Reagents Required

Refer to the list below for additional reagents and equipment required

- Absolute ethanol
- Centrifuge tubes and centrifuge for harvesting up to 4 ml bacterial culture
- Standard tabletop microcentrifuge capable of 13,000 × *g* centrifugal force (*e.g.,* Eppendorf 5415C or equivalent)
- Microcentrifuge tubes, 1.5 ml, sterile

Application

Isolation of up to 15 µg purified plasmid DNA from bacterial cultures, which may be used directly in downstream applications such as restriction enzyme digestion, PCR, cloning, sequencing, *in vitro* transcription, or labeling reactions.

The Elution Buffer is now 10 mM Tris, pH 8.5 which is the optimum sample buffer for subsequent applications.

The procedure can be adapted to purify larger quantities of plasmid DNA.

Assay Time

Total time required for preparation of 24 plasmid samples is approx. 30 min or less.

2. How To Use this Product

2.1 Before You Begin

Precautions

⚠ Binding Buffer and Wash Buffer I contain guanidine hydrochloride which is a chemical hazard and irritant.

- Do not let these buffers touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.
- Never store or use the Binding Buffer and Wash Buffer I near human or animal food.
- Always wear gloves and follow standard safety precautions when handling these buffers.

Sample Material

0.5 – 4.0 ml *E. coli* cultures (at a density of 1.5 - 5.0 A_{600} units per ml)

Bacterial cultures should be grown for 12 to 16 hours, in fluid medium (*e.g.,* LB) containing a selective antibiotic, to a density of 1.5 to 5.0 A_{600} units/ml (4).

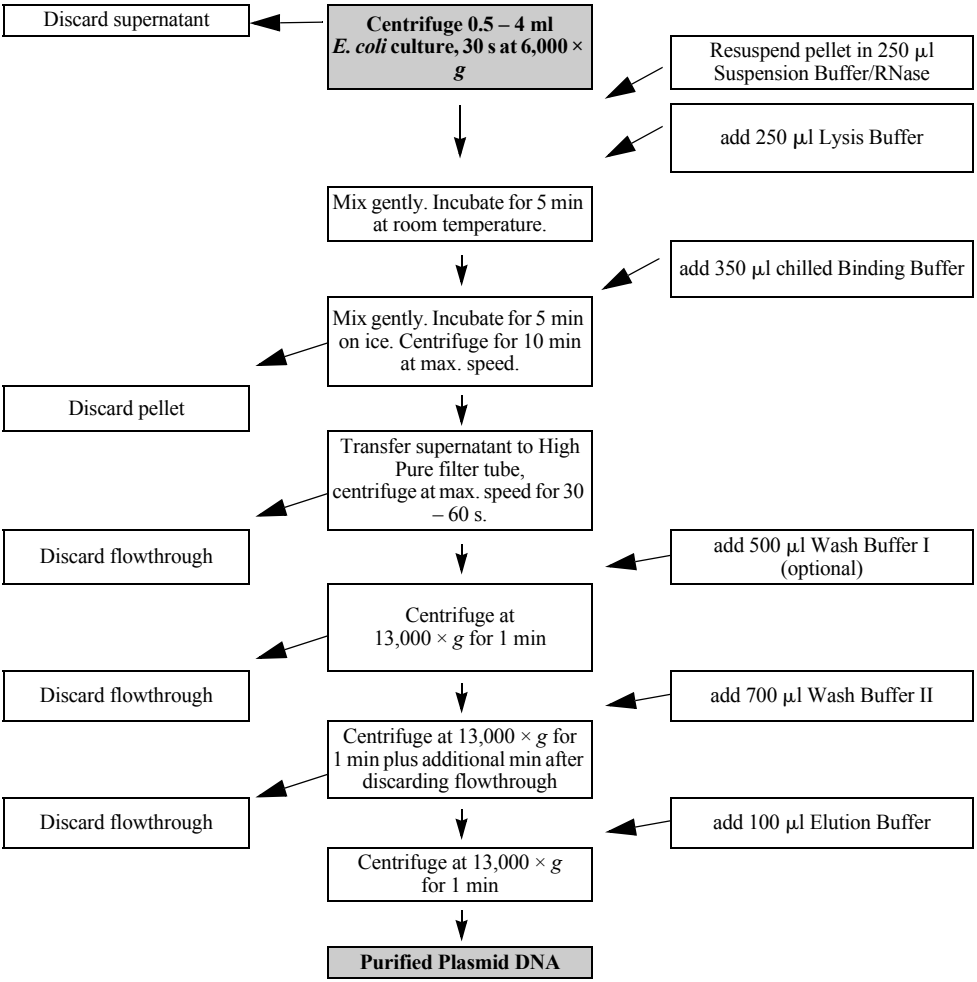
⚠ Do not use more highly concentrated samples, since these will overload the High Pure filter tube and produce unsatisfactory yields.

Preparation of Working Solutions

Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solutions:

Content	Reconstitution/ Preparation	Storage and Stability	For use in
RNase/ Suspension Buffer (Vial 1/1a; white cap)	❶ Pipette 1 ml of Suspension Buffer (bottle 1) into the glass bottle (1a) that contains lyophilized RNase. ❷ Insert a stopper and invert the vial until all the lyophilizate (including any stuck to the rubber stopper) is dissolved. ❸ Transfer all the reconstituted RNase back into the Suspension Buffer (bottle 1) and mix thoroughly.	Store the reconstituted mixture (enzyme and buffer) at +2 to +8°C; stable for 6 month.	Step 1: Removes RNA
Wash Buffer I (Vial 4, black cap)	Add 20 ml (60 ml) absolute ethanol to Wash Buffer I before using it for the first time.	Store at +15 to +25°C. Stable until expiration date printed on kit label.	Step 6 (optional): Removes nucleases
Wash Buffer II (Vial 5, blue cap	Add 40 ml (200 ml) absolute ethanol to Wash Buffer II before using it for the first time.		Step 7: Purifies the plasmid DNA from residual impurities

2.2 Experimental Overview Isolation Protocol



Protocol for preparing DNA from 0.5 - 4.0 ml of *E. coli* culture with a density of 1.5-5.0 A_{600} units per ml.

Scaling up to 10 ml is possible, nothing has to be modified in the protocol, the volumes of the solutions stay the same, as higher volumes would affect the capacity of the columns. The yield depends on the growing conditions of the strain and the lysis efficiency as seen in the table of experimental results.

⚠ You must place the Binding Buffer on ice before starting the procedure.

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 µl 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 µl 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 any temperature between +15 and +25°C.

  ⚠ Do not incubate for more than 5 min!

4 Treat the lysed solution as follows:
- Add 350 µl 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.

Centrifuge for 10 min at approx. 13,000 × *g* (full speed) in a standard tabletop microcentrifuge

5 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 1 min at full speed.

❻ After centrifugation:
- Remove the Filter Tube from the Collection Tube, discard the flowthrough liquid, and re-insert the Filter Tube in the same Collection Tube.
- If the E. coli strain in Step 2 has a high nuclease content (*e.g.*, HB101 or JM strains), perform the optional wash step below before going to Step 7.
  If the E. coli strain in Step 2 does not have a high nuclease content (*e.g.*, XL1 blue or DH5 strains), skip the optional wash step and perform Step 7.

Optional wash step: To eliminate high nuclease activity from the preparation:
- Add 500 µl of Wash Buffer I to the upper reservoir of the Filter Tube.
- Centrifuge for 1 min at full speed and discard the flowthrough.

❼ To wash the preparation:
- Add 700 µl Wash Buffer II to the upper reservoir of the Filter Tube.
- Centrifuge for 30 - 60 s at full speed and discard the flowthrough.

❽ After discarding the flowthrough liquid:
- Centrifuge the entire High Pure tube assembly for additional 1 min.
- Discard the Collection Tube.

The extra centrifugation time ensures removal of residual Wash Buffer.

❾ To elute the DNA:
- Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube.
- Add 100 µl 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 1 min at full speed.

❿ The microcentrifuge tube now contains the eluted plasmid DNA.

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.

3. Results

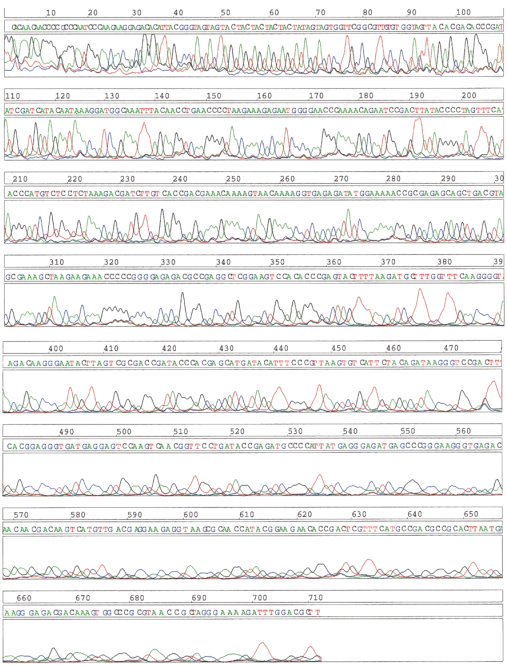
Automated Sequencing of Plasmid DNA

When a template was prepared with the High Pure Plasmid Isolation Kit, a LI-COR 4000 L automated sequencing system could read more than 700 nucleotides from that template in Auto Read mode.

Initial denaturation, 2 min at +95°C; then, repeated cycles of denaturation (30 s, +95°C), and extension/termination (60 s, +70°C). Aliquots (2 µl) of the sequen-cing mixture were applied to a 4.3% PAA gel (66 cm plate). The sequence was analyzed with a LI-COR Automated DNA Sequencer (Model 4000L) 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 4000 L automated sequencing system in Auto Read mode.



Other automated sequencing system have been tested on templates that were prepared with the High Pure Plasmid Isolation Kit. The results were similar to those above.

4. Troubleshooting

	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 after you receive it.
	Buffers or other reagents were exposed to conditions that reduced their effectiveness.	• Store all buffers at +15 to +25°C. • After reconstitution of RNase with Suspension Buffer store aliquots at +2 to +8°C. • Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination.
	Ethanol not added to Wash Buffer.	• Add absolute ethanol to all Wash Buffers before using. • After adding ethanol, mix the Wash Buffer well and store at +15 to +25°C. • Always mark Wash Buffer vial to indicate whether ethanol has been added or not.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Low recovery of nucleic acids after elution	Elution buffer has a neutral or acidic pH.	• Do not use water to elute nucleic acids from Filter Tube. Alkaline pH is required for optimal elution. • Use the Elution Buffer in the kit incomplete or no restriction enzyme cleavage of product.
Incomplete or no restriction enzyme cleavage of product	Glass fibers, which can coelute with the nucleic acid, may inhibit enzyme reactions.	1. After elution step is complete, remove High Pure filter from tube containing eluted sample and spin this sample tube for 1 minute at maximum speed. 2. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Absorbance (A_{260}) reading of product too high	Glass fibers, which can coelute with nucleic acid, scatter light.	See suggestions under "Incomplete or no restriction enzyme cleavage of product" above.
Sample "pops" out of wells in agarose gels	Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer.	1. After the last wash step, make certain flowthrough solution containing Wash Buffer does not touch the bottom of the High Pure Filter Tube. 2. If this has occurred, empty the Collection Tube, reinsert the contaminated filter, and recentrifuge for 30 seconds.

	Possible Cause	Recommendation
Low plasmid yield	Too few cells in starting material.	Grow *E. coli* to an absorbance (A_{600}) of 1.0–1.9 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 Binding Buffer is added to the lysate. The precipitate should pellet completely during centrifugation.
	Lysate did not bind completely to High Pure Filter Tube.	Pre-equilibrate the glass fiber fleece in the Filter Tube by adding 200 µl Binding Buffer to the Filter Tube before applying sample. (If you want to increase your yield in the standard protocol, always perform this extra pre-equilibration step.) ⚠ Do not centrifuge the Filter Tube after this step. Instead apply the sample (containing 350 µl Binding Buffer) to the filter tube, mix by inversion, incubate on ice for 5 min, then centrifuge as directed in step 5 of the protocol.
Plasmid is degraded or no plasmid is obtained.	High levels of nuclease activity.	Use optional Wash Buffer I (step 6 of protocol) to eliminate nuclease activity in *E. coli* strains with high levels of nuclease (for example, HB101).
RNA present in final product.	RNase not completely dissolved.	Follow the instructions given under "Preparation of Working Solutions". 🔍 Reconstituted mixture is stable for 6 months when stored properly.
	Too many cells in starting material.	Do not use more than 4 ml of an overnight *E. coli* culture as starting material.
Additional band, running slightly faster than supercoiled plasmid, is seen on gels.	Denatured plasmid in final product.	Reduce the incubation time during step 3 (lysis step) of the protocol.

5. Additional Information on this Product

How this Product Works

The High Pure Plasmid Isolation Kit relies on an alkaline lysis to free the plasmid DNA from the cell, leaving behind the *E. coli* chromosomal DNA trapped in the cell wall debris. After the solution is cleared of cell debris and chromosomal DNA, the supernatant is retained and passed to the spin Filter Tube. The nucleic acid binds specifically to the surface of glass fibers in the presence of chaotropic salt (guanidine HCl). Since the binding process is specific for nucleic acids, the bound plasmid DNA is purified from salts, proteins and other cellular impurities by washing steps followed by elution in low-salt buffer or water.

The High Pure Plasmid Isolation Kit

- saves time, because the kit can prepare up to 24 plasmid samples in less than 30 min, with minimum hands-on-time required
- minimizes DNA loss, because the kit removes contaminants without precipitation or other handling steps that can lead to lost or degraded DNA
- increases lab safety, because the kit does not use hazardous organic reagents such as cesium chloride, phenol, chloroform, or ethidium bromide
- improves reliability and reproducibility of downstream procedures, because the kit removes RNA and other impurities that might cause the plasmid DNA to behave unpredictably.

Test Principle

① Bacterial *E. coli* cells are lysed according with alkali. Simultaneously bacterial RNA is removed by treatment with RNase A.
② Lysate is neutralized and high salt is added to establish DNA binding conditions.
③ Chromosomal DNA is precipitated with cellular debris and separated by centrifugation. The supernatant contains the plasmid DNA.
④ Plasmid DNA is bound to the glass fibers pre-packed in the High Pure Filter Tube.
⑤ Bound plasmid DNA is washed to remove contaminating bacterial components.
⑥ Purified plasmid DNA is recovered using the Elution Buffer.

Product Characteristics

Purity Plasmid DNA is free of all other bacterial components, including RNA.

Yield Yield is variable and depends both on the particular *E. coli* strain used and the cell density of the bacterial culture.

Dependence of Plasmid Yield (pUC19) from *E. coli* strain used

Culture	Volume			
E.coli host strain/ densitiy	0.5 ml	1.0 ml	2.0 ml	4.0 ml
XL1 blue (3.6 A_{600} units/ml)	4.9 µg	8.6 µg	11.8 µg	14.6 µg
DH5 alpha (1.5 A_{600} units/ml)	0.9 µg	1.7 µg	3.3 µg	6.2 µg
HB101 (4.7 A_{600} units/ ml)	1.8 µg	3.5 µg	5.9 µg	8.2 µg

Dependence of Plasmid Yield (pUC19) from Cell Densitiy (2 ml *E. coli* HB101 suspension)

Culture	Volume				
Cell densitiy (A_{600} units/ ml)	0.4	0.8	1.4	3.4	5.6
Plasmid yield	0.3 µg	0.5 µg	0.8 µg	4.0 µg	6.9 µg

References

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Quality Control

Plasmid pUC19 (4.5 μg) is purified from a 1.5 ml suspension of *E. coli* JM83, which was grown in LB-medium with ampicillin for 16 hours (to a cell density of 5 A_{600} units/ml). 1 μg of the purified plasmid DNA is incubated for 1 hour at +37°C with 5 units of the restriction endonuclease *Eco RI* and then analyzed by agarose gel electrophoresis. The isolated plasmid DNA is as sensitive to restriction endonuclease digestion as plasmid DNA isolated by CsCl density centrifugation.

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 Listing using the number symbol type 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.

6.2 Changes to Previous Version

- Editorial changes

6.3 Ordering Information

Roche 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

Associated Kits

Product	Pack Size	Cat. No.
Genopure Plasmid Midi Kit	1 kit (for up to 20 preparations)	03 143 414 001
Genopure Plasmid Maxi Kit	1 kit (for up to 10 preparations)	03 143 422 001

6.4 Trademarks

6.5 Regulatory Disclaimer

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