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Not for use in diagnostic procedures.

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High Pure Plasmid Isolation Kit

Version: 11
Content version: September 2017

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

Cat. No. 11 754 777 001	1 kit 50 purifications
Cat. No. 11 754 785 001	1 kit 250 purifications

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	white	Suspension Buffer	Contains 50 mM Tris-HCl and 10 mM EDTA, pH 8.0 (+25° C)	11754777001	▪ 25 ml
				11754785001	▪ 80 ml
1a	white	RNase A	Dry powder to be dissolved in Suspension Buffer	11754777001	▪ 2.5 mg
				11754785001	▪ 8 mg
2	red	Lysis Buffer	Contains 0.2 M NaOH and 1% SDS	11754777001	▪ 25 ml
				11754785001	▪ 80 ml
3	green	Binding Buffer	Contains 4 M guanidine hydrochloride and 0.5 M potassium acetate, pH 4.2	11754777001	▪ 25 ml
				11754785001	▪ 100 ml
4	black	Wash Buffer I	For performing an optional wash step of strains with high nuclease activity (e.g., E. coli HB101) Contains 5 M guanidine hydrochloride, 20 mM Tris-HCl, pH 6.6 (+25°C) (final concentrations after addition of ethanol)	11754777001	▪ 33 ml (100 ml), add 20 ml (60 ml) absolute ethanol
				11754785001	▪ 100 ml, add 60 ml absolute ethanol
5	blue	Wash Buffer II	For strains with low nuclease activity Contains 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) (final concentrations after addition of ethanol)	11754777001	▪ 10 ml (50 ml); add 40 ml (200 ml) absolute ethanol
				11754785001	▪ 50 ml; add 200 ml absolute ethanol
6	colorless	Elution Buffer	Contains 10 mM Tris-HCl, pH 8.5 (+25°C)		▪ 40 ml
7		High Pure Filter Tubes	For processing up to 700 µl sample volume	11754777001	▪ One bag containing 50 polypropylene tubes with two layers of glass fiber fleece
				11754785001	▪ Five bags containing 50 polypropylene tubes with two layers of glass fiber fleece
8		Collection Tubes		11754777001	▪ One bag containing 50 polypropylene tubes with two layers of glass fiber fleece
				11754785001	▪ Five bags containing 50 polypropylene tubes with two layers of glass fiber fleece

Both pack sizes of the kit contain the same components; they differ only in how much of each component they contain.

All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.

The buffers can show a slight yellow color. This will have no impact on the function of the buffer.

1.2. Storage and Stability

Storage Conditions (Product)

⚠ ***The High Pure Plasmid Isolation 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.***

⚠ ***Improper storage 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 impact the performance of the kit.***

Storage Conditions (Working Solution)

Solution	Storage
Suspension Buffer	+2 to + 8°C

1.3. Additional Equipment and Reagents 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

1.4. Application

Isolation of up to 15µg 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.*

1.5. Preparation Time

Total time	Approx. 30 minutes for 24 plasmid samples

2. How to Use this Product

2.1. Before you Begin

Sample Materials

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 – 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.***

General Considerations

Handling Instructions

⚠ ***Guanidine-hydrochloride in Binding Buffer and Wash Buffer I is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
⚠ ***Do not allow 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 near human or animal food.***
⚠ ***Always wear gloves and follow standard safety precautions when handling these buffers.***
⚠ ***Do not allow the Binding or Wash buffers to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR setup and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

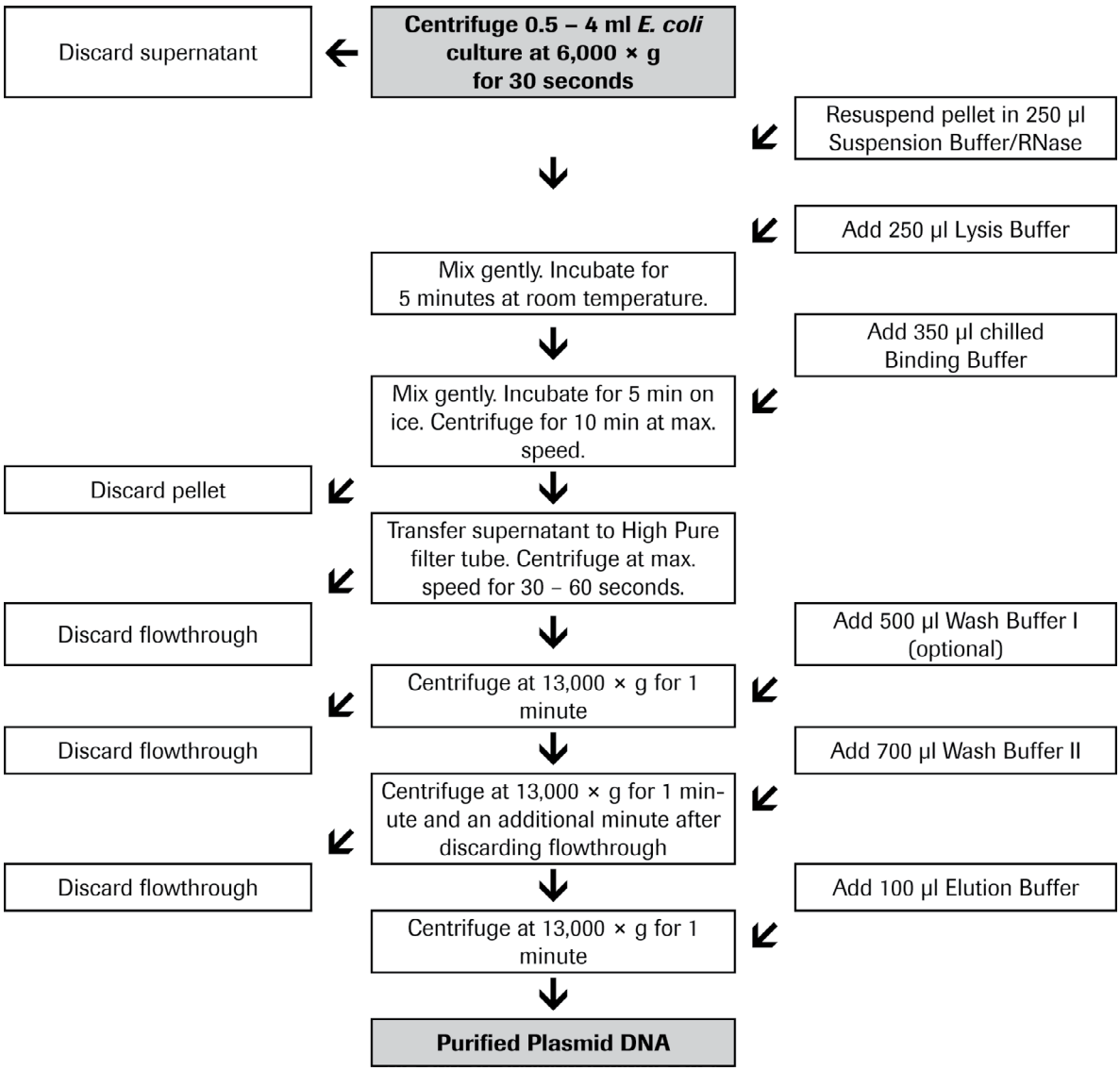
Working Solution

In addition to the ready-to-use solutions supplied with this kit, prepare the following working solutions:

Content	Reconstitution / Preparation	Storage / Stability	For use in...
RNase / Suspension Buffer (Vial 1 / 1a; white cap)	① Pipette 1 ml of Suspension Buffer (Bottle 1) into the glass bottle (Bottle 1a) that contains lyophilized RNase. ② Insert a 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 (Bottle 1) and mix thoroughly.	▪ Store the reconstituted mixture (enzyme and buffer) at +2 to +8°C ▪ Stable for 6 months.	Step 1: Removes RNA
Wash Buffer I (Vial 4, black cap)	11754777001: Add 20 ml absolute ethanol to Wash Buffer I before using it for the first time. 11754785001: Add 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)	11754777001: Add 40 ml absolute ethanol to Wash Buffer II before using it for the first time. 11754785001: Add 200 ml absolute ethanol to Wash Buffer II before using it for the first time.	▪ Store at +15 to +25°C. ▪ Stable until expiration date printed on kit label.	Step 7: Purifies the plasmid DNA from residual impurities

2.2. Protocols

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.

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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.

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❸ 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 minutes at any temperature between +15 and +25°C.
⚠ ***Do not incubate for more than 5 minutes.***

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❹ Treat the lysed suloution as follows:
– Add 350 µl chilled Binding Buffer.
– Mix gently by inverting the tube 3 to 6 times.
– Incubate on ice for 5 minutes.

⚠ ***The solution should become cloudy and a flocculant precipitate should form.***
– Centrifuge for 10 minutes at approx. 13,000 × *g* (full speed) in a standard tabletop microcentrifuge

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❺ 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 minute at full speed.

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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 minute at full speed and discard the flowthrough.

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

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

ⓘ *The extra centrifugation time ensures removal of residual Wash Buffer.*

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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 minute at full speed.

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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 –15 to –25°C for later analysis.*

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3. Results

Expected 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 density (A600 units/ml)	0.4	0.8	1.4	3.4	5.6
Plasmid yield	0.3 µg	0.5 µg	0.8µg	4.0 µg	6.9 µg

Purity

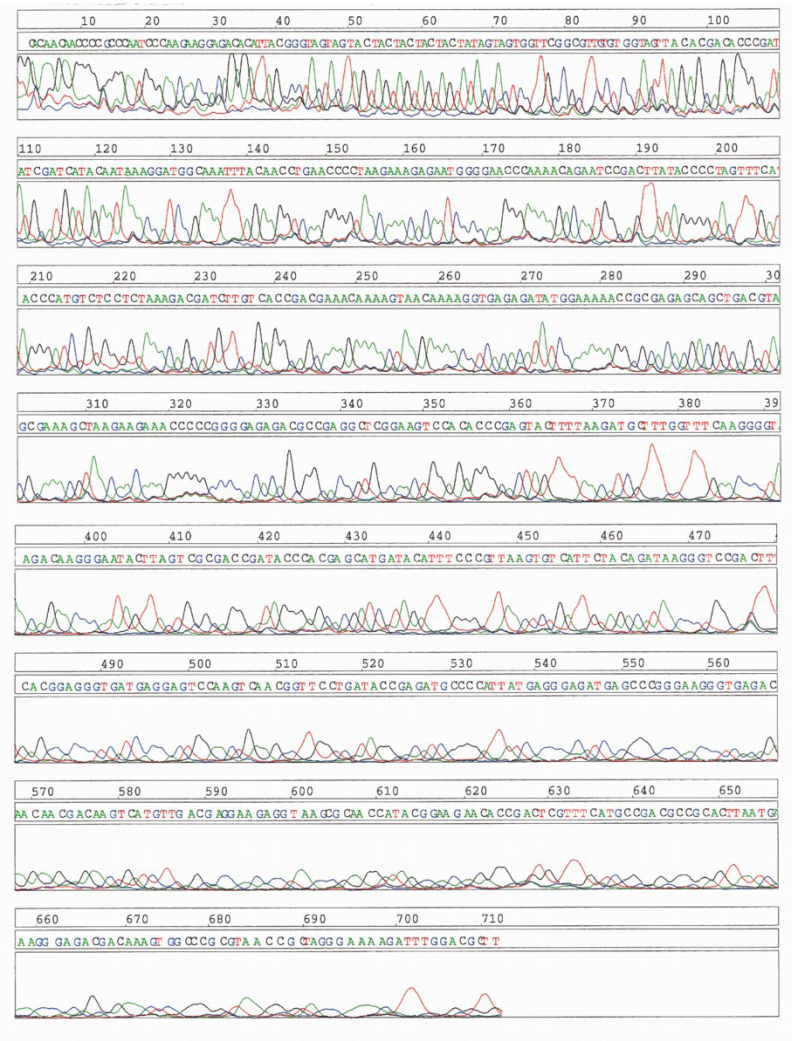
Plasmid DNA is free of all other bacterial components, including RNA.

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 can read more than 700 nucleotides from that template in Auto Read mode. Initial denaturation, 2 minutes at +95°C; followed by repeated cycles of denaturation (30 seconds at +95°C), and extension / termination (60 seconds at +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 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 systems have been tested on templates that were prepared with the High Pure Plasmid Isolation Kit. The results were similar to those above.

4. Troubleshooting

Observation	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 condi-tions 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 co-elute with the nucleic acid, may inhibit enzyme reactions.	① After elution step is finished, 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 co-elute 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 re-centrifuge for 30 seconds.

Observation	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 minutes, 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

5.1. Test Principle

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 minutes, 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.

① Bacterial E. coli cells are lysed with alkali. Bacterial RNA is simultaneously 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.

5.2. 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 *EcoR* I 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

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Trademarks

HIGH PURE is a trademark of Roche.
All third party product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

6.5. Regulatory Disclaimer

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

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country to display country-specific contact information.

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