DNAbioTech
Life Science

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High Pure Plasmid Isolation Kit

Catalog no.: DB9815

(20, 50 and 100 prep)

Intended for research use only

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Diba NoAvaran Azma Company

Customer and technical support

If you have any question, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

About this user manual

It is recommended for the first time users to read the detailed protocol sections of the user manual before using these products. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-aglance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

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Contents

General description

The DNAbiotech high pure plasmid extraction kit is designed to plasmid DNA from an overnight culture of bacteria transformed with a plasmid, with a total biomass (O.D.600 of culture × volume of culture in μl) of 0.5 -4 ml. When working with low-copy-number plasmids, more culture volumes are recommended.

This system can be used to isolate any plasmid from *E. coli* hosts. Purified plasmid can be used without further manipulation for automated fluorescent DNA sequencing as well as for other standard molecular biology techniques.

Technical Bulletin describes isolation of plasmid DNA from *E. coli*. Plasmid yield will vary, depending on a number of factors, including culture volume, plasmid copy number, type of culture medium and bacterial strain used.

Kit specifications

- DNAbiotech high pure plasmid extraction kit is designed for the rapid isolation of highly pure Plasmid from **an overnight culture of bacteria.**

-The obtained plasmid is ready-to-use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reactions.

Quality Control

In accordance with DNAbiotech Co. Management System, each part of the **high pure plasmid extraction kit** is tested against predetermined specifications to ensure consistent product quality**.**

Safety Notes

The buffers included in **high pure plasmid extraction kit** contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions.

Buffers contain chaotropes agents. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste**.**

Elution procedures

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8. This will increase DNA stability especially during long term and / or multi use storage at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications. For optimal performance of isolated DNA in subsequent downstream applications we recommend elution with the supplied elution buffer and storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Kit Components

No.	Name	cat #: DB9815-20rxn	cat #: DB9815-50rxn	cat #: DB9815-100 rxn
1	*Handbook protocol*	1	1	1
2	Columns and Collection Tubes (pcs)	20	50	100
3	Suspension Buffer	8 ml	15 ml	30 ml
4	RNase A * (Lyophilized)	As need	As need	As need
5	Lysis Buffer	8 ml	15 ml	30 ml
6	Binding Buffer	10 ml	20 ml	40 ml
7	WB1 (Concentrate)**	9ml (add 6 ml absolute ethanol)	18ml (add 12 ml absolute ethanol)	2 × 18ml (add 2 × 12 ml absolute ethanol)
8	WB2 (Concentrate)**	4 ml (add 16 ml absolute ethanol)	8 ml (add 32 ml absolute ethanol)	2 ×8 ml (add 2 × 32 ml absolute ethanol)
9	Elution buffer (EB)	5 ml	10 ml	20 ml

* RNase A should be mix with Suspension Buffer and then stored at 4° C for up 6 months.

** 96-100 % pure ethanol should be added.

Note: During storage, especially at low temperatures, a precipitate may form in some Buffers. Such precipitates can be easily dissolved by incubating the bottle at 60 °C.

Storage condition:

Shipping: RT

Storage: 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 4° 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 isolationkits are always shipped at ambient temperature.

Before experiment notes:

Sample Material

- 0.5 – 4.0 ml E. coli cultures (at a density of 1.5 - 5.0 A600 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 A600 units/ml.
- Do not use more highly concentrated samples, since these will over loadthe 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:

- Pipette 1 ml of Suspension Buffer into the microtube that contains lyophilized RNase, invert vial until all the lyophilized is dissolved. Then transfer all the reconstituted RNase back into the suspension buffer and store this mixture at +2 to + 8˚C for 6 month.
- Add absolute ethanol to Wash Buffer I (According to the table) before using it for the first time. Store at +15 to +25˚ C . Stable until expiration date printed on kit label.
- Add absolute ethanol to Wash Buffer II (According to the table) before using it for the first time. Store at +15 to +25˚ C . Stable until expiration date printed on kit *label*

Protocol

Protocol **is** for preparing **plasmid** from 0.5 - 4.0 ml of E. coli culture **with a density of 1.5-5.0 A600 unit** per ml.

Scaling up to 10 ml is possible, nothing has to be modified in the protocol, and 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- Pellet the bacterial cells from 0.5 - 4.0 ml of (overnight, 12-16 hours) E. coli culture. The cells should have a density of 1.5 - 5.0 A 600 units per ml.

Discard the supernatant.

Note: the pellet could be store at – 20^{o} C for 15 min, it increase the efficacy of lysis step.

2- Add 250 µl Suspension Buffer +RNase A to the centrifuge tube containing the bacterial pellet. Resuspend the bacterial pellet and mix well by vortex.

3- Add 250 µl Lysis Buffer. Mix gently by inverting the tube 3 to 6 times.

Note1: To avoid shearing genomic DNA, do not vortex!

Incubate for 5 min on ice.

Note2: Do not incubate for more than 5 min!

4- 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 flocculent precipitate should form.

5- Centrifuge for 10 min at approx. 12,000 × g.

6- Insert a Filter column into one Collection Tube. Transfer entire supernatant from Step 5 into column. Insert the column and collection tube assembly into the microcentrifuge and centrifuge for 1 min at full speed.

7- Remove the column from the Collection Tube, discard the flowthrough liquid, and re-insert the Filter Tube in the same Collection Tube.

8- Add 500 µl of Wash Buffer I to the upper reservoir of the column. Centrifuge for 1 min at full speed and discard the flowthrough.

Note1: This step is optional (better to do) for strains which have high nuclease content (e.g., HB101 or JM strains). This step may reduce the final concentration of plasmid although you will get more pure plasmid.

9- Add 700 µl Wash Buffer II to the upper reservoir of the column. Centrifuge for 30 - 60 s at full speed and discard the flowthrough.

10- Centrifuge the column and collection tube for additional 1 min. The extra centrifugation time ensures removal of residual Wash Buffer.

11- Discard the Collection Tube.

12- Insert the Filter Tube into a clean, sterile 1.5 ml microcentrifuge tube. Add about 100 µl of preheated Elution Buffer or DW and heat tube assembly at 65°C for 1 min. Centrifuge the tube assembly for 1 min at full speed.

13- 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.

14- Apply 4-5 µl of eluted plasmid into agarose gel.

Troubleshooting

	Possible Cause	Recommendation
Low plasmid yield	Too few cells in starting material.	Grow E. coli to an absorbance (A600) 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, and 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 is seen on gels	Denatured plasmid in final product.	Reduce the incubation time during step 3 (lysis step) of the protocol

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- ✓ Taq polymerase and PFU master mix
- ✓ Molecular grade buffers (TAE, TBE, RIPA and….)
- ✓ Column based DNA/RNA extraction kits.
- ✓ And ….

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