NucleoGene Plasmid Mini Extraction Kit

Instructions For Use

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REF

For isolation and purification of Deoxyribonucleic acid from bacteria samples.
For research implementation only.
Not suitable for diagnostic use.
For professional use only.

NGE017– 50 Test
100 Test
250 Test

Kit Contents

	Supplied Material	50 Tests	100 Tests	250 Tests
1.	Lysis Buffer†	10 mL	20 mL	50 mL
2.	Wash Buffer I*	15 mL	30 mL	75 mL
3.	Wash Buffer II*	5 ml	10 mL	25 mL
4.	Binding Buffer	20 mL	40 mL	100 mL
5.	Elution Buffer	6 mL	12 mL	30 mL
6.	Suspension Buffer	10 mL	20 mL	50 mL
7.	Spin Column	50	100	250
8.	Collection Tubes	50	100	250
9.	User's Guide	1	1	1

*Look at''Notes Before Starting'' for preparation of Wash Buffers.

†Precipitation may occur during storage. Heat to dissolve the precipitate formed before use.

Storage Conditions & Robustness

Kit components should be stored at room temperature (15-25°C) excluding Suspension Buffer. Do not expose Kit to direct sunlight. Suspension Buffer should be stored at +4 °C. When used under the seconditions, the kit can be stored for 12 months without any performance loss.

Intended Use

NucleoGene Plasmid Mini Extraction Kit includes spin column and special lysis buffer system for effectively RNA free plasmid DNA extraction from bacteria samples. Specifically designed for DNA isolation and purification. Suitable for downstream applications such as purified DNA, PCR, Real Time Qualitative and Quantitative PCR (qPCR) and microarray. NucleoGene Plasmid Mini Extraction Kit is designed for research use only. It is not for diagnosis and treatment of disease.

Product Usage Limits

- NucleoGene Plasmid Mini Extraction Kit is for purification of RNA free plasmid DNA from bacterial samples. It cannot be used for clinical purposes. Whether the kit is suitable for the user's own experimental design is up to the user's discretion.
- In particular, the ability of this kit to isolate plasmid DNA from bacteria samples has been confirmed.
- The collection, transport and storage of the sample to be used are at least important as purification andare sensitive stages that may affect the results.
- It is designed for professional use by educated personnel only.
- Kits or any kit components with different lot numbers should not be used together.

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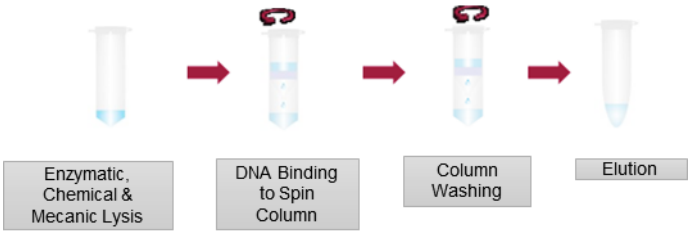
Introduction

NucleoGene Plasmid Mini Extraction Kit is improved for RNA free plasmid DNA extraction effectively from bacteria samples to be a quick and useful method. Working principle of Kit depends on binding of DNA with its especially improved solutions to spin columns. The superior performance of the kit is due to its ability to lyse the cell walls of bacteria samples, as well as its ability to remove PCR inhibitors present in the sample with solutions specially improved for bacteria samples. While wash buffer optimized specifically is improved for purified nucleic acids especially from protein and other inhibitors, the Lysis Buffer solution is improved for lysing the cell wall and remove the inhibitors from the sample. With the complete the isolation process, DNA is obtained at high efficiency and purity.



NucleoGene Plasmid Mini Extraction Kit is optimized for use in 0.5 – 4.0 ml of E. coli culture with a density of 1.5 – 5.0 A600 units per ml sample. Purified DNA with completed isolation should be used immediately or a freezer at -20 ° C should be preferred for long-term storage.

Warnings & Precautions

- All clinical specimens and residues and debris arising therefrom must be treated as potential infectious agents and disposed of accordingly.
- All samples should be prepared in Biosafety Level 1 and 2 or Class II Biosafety Cabins.
- All surfaces should be cleaned daily with a disposable paper towel or napkin by using freshly prepared Sodium Hypochlorite %20 Solution.
- Do not neglect to use laboratory safety consumable materials such as disposable gloves, goggles, visor, disposable cuffs, and disposable mask.
- If any of the kit components come into contact with your skin, wash it with plenty of water immediately. In case any of the kit components come into contact with your mucous membranes such as your eyes or mouth, wash the contact part with plenty of water again; and go to the doctor. If possible, prefer filtered pipette tips.
- Do not mix the kit solutions or waste appeared during isolation with bleach.
- Keep Kit out of contamination sources such as DNA and RNA, especially amplified nucleic acid.
- Do not mix solutions with different lot numbers or do not use or combine products from other companies.
- For more information, please apply the Material Safety Data Sheet(MSDS), which you can request from www.nucleogene.com.

Other Required Materials

- Thermal Blockorwater bath
- Ethanol (%96-100)
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Mikrocentrifuge tubes (2.0 ml)
- Micropipette set and sterile filtered micropipette tips
- Spatula

Notes Before Starting

If the kit is kept in a non-air-conditioned environment, Lysis buffer solution can precipitate at room temperature in cold weather. In such a case to dissolve the precipitate, the Lysis buffer solution is held at 50°C in a water bath until dissolving completely. Be sure of all solutions are at the room temperature before and after protocol implementation, if they are not, allow them to reach room temperature.

Wash Buffer I

Add 10 mL of 96 % or absolute ethanol Wash I Buffer bottle for 50 Test

Add 20 mL of 96 % or absolute ethanol Wash I Buffer bottle for 100 Test

Add 50 mL of 96 % or absolute ethanol Wash I Buffer bottle for 250 Test

Wash Buffer II

Add 20 mL of 96 % or absolute ethanol Wash II Buffer bottle for 50 Test

Add 40 mL of 96 % or absolute ethanol Wash II Buffer bottle for 100 Test

Add 100 mL of 96 % or absolute ethanol Wash II Buffer bottle for 250 Test

Then, do not forget shaking with your hand or with vortex at least 10 seconds.

Protocol

Control the reagents

Before starting to DNA isolation, control reagents that are free of leakage. To mix the solutions, shake the reagents slightly. If rea-gents precipitate (specially Lysis solution), they dissolve at 50 ° C by heating.

Plasmid Isolation

1) Transfer 1.5 mL from a fresh overnight culture to a microcentrifuge tube. To pellet bacteria, centrifuge at full speed (16.000 × g) in a microcentrifuge for 30 seconds. Discard supernatant and re centrifuge. Remove any residual supernatant using a pipette.

*If processing 3 mL culture volumes, repeat step 1. Pelleted DNA can be stored at -20° C if necessary.

Note:
If purifying a high molecular weight or low copy number plasmid, process 3 mL culture volume.

2) Add 175 µL Suspension Buffer to the bacterial pellet and thoroughly re-suspend the pellet.

Note:
Cell re-suspension can be achieved by either vortexing, pipetting up and down or by scraping the base of the microcentrifuge tube across the surface of an empty pipette tip box. Incomplete cell re-suspension will result in reduced plasmid DNA recovery.

3) Add 175 µL Lysis Buffer and mix immediately by gentle inversion (approximately 5 times) until solution becomes clear and viscous.

Note:
Vigorous mixing will shear genomic DNA resulting in contamination of the final purified sample. Do not vortex. Do not allow the lysis reaction to exceed 5 minutes. Lysis Buffer contains NaOH which will denature the plasmid DNA on prolonged incubation.

4) Add 350 µL Binding Buffer and mix immediately by gentle inversion until the precipitate is evenly dispersed.

Note:
Cellular debris, genomic DNA and KDS appear as a white flocculent precipitate. Continue gentle inversion until the precipitate is evenly dispersed (approximately 10 times). The precipitate must be effectively dispersed to ensure consistent purity and yield of the isolated plasmid DNA. Do not shake or mix vigorously since genomic DNA will be sheared and co-purify with the plasmid DNA; mix by gentle inversion.

5) Centrifuge at full speed (approximately 16000 × g) for 4 minutes.

Note:
For applications that require less stringent purity (e.g. DNA sequencing) a 3 minute flocculent spin can be performed. For applications that are more salt sensitive, a > 5 minute flocculent spin may be required.

6) During centrifugation, for each purification that is to be performed, place one NucleoGene Plasmid Mini column in one Collection tube.

7) Carefully transfer the cleared supernatant to the NucleoGene mini spin column (approximately 700 µL). Close the lid of the column gently.

8) Centrifuge at full speed (approximately 16000 × g) for 30 seconds. Discard the flowthrough by emptying the Collection tube.

Note:

When purifying plasmid DNA from 3 mL culture volumes, the user may notice some residual lysate on the column after this centrifugation step. This can be ignored; proceed with the Wash & Dry step below. On washing this residual lysate will generally pass through the column with no effect on the yield or purity of the isolated plasmid DNA.

If significant volumes of residual lysate do remain, this probably indicates that excessive flocculent material is being transferred onto the NucleoGene Plasmid Mini Column, blocking the silica membrane. Increase the time of the flocculent spin above to 6–10 minutes, providing that the culture A600 was approximately 2.5.

Note:

It is recommended that a pipette is used but decanting directly can be performed as an alternative. It is important to avoid transferring any cellular debris to the column as this will affect the purity of the isolated plasmid DNA.

9) Wash the column with 400 µL Wash I Buffer and centrifuge at full speed (approximately 16000 × g) for 30 seconds. Discard the flowthrough.

10) Add 400 µL Wash II Buffer to the column and centrifuge at full speed (approximately 16000 × g) for 1 minute. Carefully discard flowthrough for in Collection tube.

Note:

After centrifugation, if any of the Wash II Buffer comes into contact with the bottom of the column, discard the flow through and re-centrifuge for 30 seconds. The presence of contaminating ethanol in the eluted plasmid DNA may affect the downstream applications and therefore care must be taken to ensure its complete removal.

11) Transfer the NucleoGene Plasmid Mini Column into a fresh 1,5-2 ml microcentrifuge tube and add 100 µL Elution Buffer directly onto the center of the column. Incubate the column for 1 min at room temperature. Microcentrifuge at full speed (approximately 16000 × g) for 1 min to recover the plasmid DNA as flowthrough in the microcentrifuge tube. Store the purified plasmid DNA at -20°C.

Note:

If a more concentrated sample is required, add single 50 µL volume of Elution Buffer, incubate for 1 minute and centrifuge to elute. If a higher yield is required, follow the elution protocol described above, but elute with two successive 50 µL elution volumes.

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Troubleshooting

Issue	Possible cause	Recommendation
Low nucleic acid yield or purity	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.
	Reagents and samples not completely mixed.	Always mix the sample tube well after addition of each reagent.
Incomplete or no restriction enzyme cleavage of product	Glass fibers, which can coelute with the nucleic acid, may inhibit enzyme reactions.	After elution step is finished, remove Spin Column from tube containing eluted sample and spin this sample tube for 1 minute at maximum speed. Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube.
Sample "pops" out of wells in agarose gels	Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer	After the last wash step, make certain flowthrough solution containing Wash Buffer does not touch the bottom of the Spin Column. If this has occurred, empty the Collection Tube, reinsert the contaminated filter, and recentrifuge for 30 seconds.
Low plasmid yield	Lysate did not bind completely to Spin Column.	Pre-equilibrate the glass fiber fleece in the Spin Column by adding 200 µl Binding Buffer to the Spin Column 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 Spin Column after this step. Instead apply the sample (containing 350 µl Binding Buffer) to the spin column, mix by inversion, incubate on ice for 5 minutes, then centrifuge as directed in Step 5 of the protocol.

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