NucleoGene PCR Product and Gel Purification Kit

Instructions For Use

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REF

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

NGE019– 50 Tests
100 Tests
250 Tests

Kit Contents

	Supplied Material	50 Tests	100 Tests	250 Tests
1.	Binding Buffer	30 mL	60 mL	150 mL
2.	Wash Buffer	10 mL	20 mL	50 mL
3.	Elution Buffer	40 mL	80 mL	200 mL
4.	Spin Column	50	100	250
5.	Collection Tubes	50	100	250
6.	User's Guide	1	1	1

Storage Conditions & Robustness

Kit components should be stored at room temperature (15-25°C) When used under the conditions, the kit can be stored for 12 months without any performance loss.

Intended Use

NucleoGene PCR Product and Gel Purification Kit includes spin column and special binding buffer system for effectively DNA purification from PCR Product and Agarose Gel. Specifically designed for DNA purification. Suitable for downstream applications such as purified DNA, PCR, Real Time Qualitative and Quantitative PCR (qPCR) and microarray. NucleoGene NucleoGene PCR Product and Gel Purification Kit is designed for research use only. It is not for diagnosis and treatment of disease.

Product Usage Limits

- NucleoGene PCR Product and Gel Purification Kit is for purification of DNA from PCR Product and Agarose Gel. 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 DNA from PCR Product and Agarose Gel 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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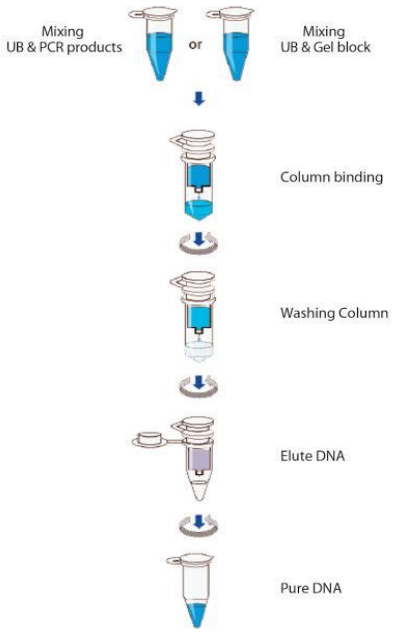
Introduction

NucleoGene PCR Product and Gel Purification Kit is improved for DNA purification effectively from PCR Product and Agarose Gel 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 binding of PCR Product and Agarose Gel, as well as its ability to remove PCR inhibitors present in the sample with solutions specially improved for PCR Product and Agarose Gel. While wash buffer optimized specifically is improved for purified DNA especially. With the complete the purification process, DNA is obtained at high efficiency and purity.



NucleoGene PCR Product and Gel Purification Kit is optimized for use in 100 mg agarose gel and 100 uL PCR Product 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)
- TAE buffer (40 mM Tris-acetate, 1 mM EDTA), pH 8.0 or
- TBE buffer (89 mM Tris-borate, 2 mM EDTA), pH 7.8
- Electrophoresis equipment
- Sterile scalpel
- Isopropanol
- Microcentrifuge
- Vortex mixer
- Microcentrifuge tubes (1.5 ml)
- Micropipette set and sterile filtered micropipette tips
- Spatula

Notes Before Starting

-Add 40 mL of 96 % or absolute ethanol wash buffer bottle in the 50 tests of kit to operationalize of Wash Buffer solution.
-Add 80 mL of 96 % or absolute ethanol wash buffer bottle in the 100 tests of kit to operationalize of Wash Buffer solution.
-Add 200 mL of 96 % or absolute ethanol wash buffer bottle in the 250 tests of kit to operationalize of Wash Buffer solution.

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, they dissolve at 50 ° C by heating.

Purification of PCR Products

1. After PCR is finished, adjust total volume for each PCR tube (reaction components + DNA product) to 100 µl:
– Add 500 µl Binding Buffer to each 100µl PCR tube.
*Mineral oil or wax does not need to be removed from the PCR solution before adding the Binding Buffer.
– Mix sample (Binding Buffer + PCR solution) well.

2. Insert one Spin Column into one Collection Tube. Transfer the sample from step 1 using a pipette to the upper reservoir of the Spin Column. Centrifuge 30 – 60 s at maximum speed in a centrifuge at +15 to +25°C.

3. Disconnect the Spin Column, and discard the flowthrough solution. Reconnect the Spin Column to the same Collection Tube.

4. Add 500 µl Wash Buffer to the upper reservoir. Centrifuge 1 min at maximum speed (as above).

5. Discard the flowthrough solution. Recombine the Spin Column with the same Collection Tube. Add 200 µl Wash Buffer. Centrifuge 1 min at maximum speed (as above).
*This second 200 µl wash step ensures optimal purity and full removal of Wash Buffer from the glass fibers.

6. Discard the flowthrough solution and Collection Tube. Reconnect the Spin Column to a clean 1.5 ml microcentrifuge tube.

7.Liquid in the tube contains DNA and ready for use. DNA is kept at -20 ℃ for long time storage.

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Purification of DNA Fragments from Agarose Gel

1. In the following table the purification procedure for DNA from a 100 mg agarose gel slice is described:

Isolate DNA band of interest electrophoretically as follows:

– Load PCR reaction mixture on a 0.8 – 2% agarose gel.

– Use 1x TAE or 1x TBE as running buffer.

– Electrophorese until DNA band of interest is isolated from adjacent contaminating fragments

2. Identify bands by staining gel with ethidium bromide or safe dye.

3. Cut desired DNA band from gel using an ethanol-cleaned scalpel or razor blade.

4. Place excised agarose gel slice in a sterile 1.5 ml microcentrifuge tube. Determine gel mass by first pre-weighting the tube, and then re-weighting the tube with the excised gel slice.

5. Add 300 µl Binding Buffer for every 100 mg agarose gel slice to the microcentrifuge tube.

6. Dissolve agarose gel slice in order to release the DNA:

– Vortex the microcentrifuge tube 15 – 30 s to resuspend the gel slice in the Binding Buffer.

– Incubate the suspension for 10 min at 56°C.

– Vortex the tube briefly every 2 – 3 min during incubation.

7. After the agarose gel slice is completely dissolved:

– Add 150 µl isopropanol for every 100 mg agarose gel slice to the tube. Vortex thoroughly.

8. Insert one Spin Column into one Collection Tube. Pipette the entire contents of the microcentrifuge tube into the upper reservoir of the Spin Column.

9. Centrifuge 60 s at maximum speed in a centrifuge at +15 to +25°C.

10. Discard the flowthrough solution. Reconnect Spin Column with the same Collection Tube. Add 500 µl Wash Buffer to the upper reservoir. Centrifuge 1 min at maximum speed (as above). Discard the flowthrough solution. Recombine Spin Column with the same Collection Tube.

11. Add 200 µl Wash Buffer. Centrifuge 1 min at maximum speed.

*This second 200 µl wash step ensures optimal purity and full removal of Wash Buffer from the glass fibers.

12. Discard the flowthrough solution and Collection Tube. Recombine Spin Column with a clean 1.5 ml microcentrifuge tube. Add 50 –100 µl Elution Buffer to the upper reservoir of the Spin Column. Centrifuge 1 min at maximum speed.

13. Liquid in the tube contains DNA and ready for use. DNA is kept at -20 ℃ for long time storage.

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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