



Gelpure

NB-60-0002

Gelpure

Cat# NB-60-0002 Size : 50 columns

Description

Neo Biotech Gelpure kit is designed for the purification of DNA from TAE/TBE agarose gels and for the direct purification of PCR products. The kit can be used to purify DNA fragments from 50 bp to 20 kb. Average recoveries range from 60 to 90% depending on the fragment size. NEO BIOTECH Gelpure purification kit utilizes a silica-gel based membrane which selectively adsorbs up to 20 µg of DNA fragments in the presence of specialized binding buffers. Soluble agarose, nucleotides, oligos (<30-mer), primer dimers, eNeo Biotech mes, mineral oil and other impurities do not bind to the membrane and are washed away. DNA fragments are then eluted off the column and can be used for downstream protocols without further processing. Binding Buffer contains a pH indicator, allowing the evaluation of optimal pH for DNA binding. The pH indicator does not interfere with DNA binding.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (20-25 °C) and are stable till the expiry date. Add 48 mL of ethanol to each bottle of Wash Buffer.

System Components

- Binding Buffer - 2 × 30 mL
- Wash Buffer (concentrate) - 12 mL
- Elution Buffer (does not contain EDTA) - 15 mL
- Neo Biotech Spin Columns - 50
- Collection Tubes (2 mL) - 50

Protocol for DNA purification from Agarose Gels

All purification steps should be carried out at room temperature.

All centrifugations should be carried out at room temperature in a table-top microcentrifuge at >12000 xg (10000-15000 rpm depending on the rotor type).

1. Excise the DNA fragment from the gel with a clean, sharp scalpel. Weight the gel slice and transfer to a 1.5 mL microcentrifuge tube.
2. Add 300 µL of Binding Buffer for each 100 mg of gel weight (example – a gel slice weighing 125 mg would require 375 µL of Binding Buffer). For high concentration gels (2.0-3.0%), 500 µL of Binding Buffer per 100 mg of agarose gel should be added. The maximum amount of gel slice per Neo Biotech spin column is 400 mg. For gel slices >400 mg use more than one column.
3. Incubate at 55-60 °C for 5-10 minutes and shake occasionally until agarose is completely dissolved.
4. Check that the colour of the mixture is yellow (similar to the colour of the Binding Buffer). If the colour of the mixture is orange or violet, add 10 µL of 3 M sodium acetate pH 5.0, and mix well.

5. (Optional) For DNA fragments <500 bp or >10 kb long add 1 gel volume of isopropanol to the sample and mix well by pipetting several times (example – a gel slice weighing 125 mg would require 125 μ L of isopropanol).
6. Load the above mixture into the Neo Biotech spin column placed into a Collection tube (2 mL).
Centrifuge for 30s to 1 minute and discard the flow-through in the collection tube. The maximum volume of the column reservoir is 700 μ L. For sample volumes of more than 700 μ L, simply load and spin again.
7. (Optional) Add 500 μ L of Wash Buffer and centrifuge for 30s to 1 minute. Discard the flowthrough in the collection tube. This step is only important if DNA is intended to be used for direct sequencing, in vitro transcription or microinjection.
8. Add 600 μ L of Wash Buffer and centrifuge for 30s to 1 minute. Discard the flow-through in the collection tube.
9. Centrifuge for 1 minute to dry Neo Biotech spin membrane of residual ethanol.
10. Place the Neo Biotech spin column into a clean 1.5 mL microcentrifuge tube. Add 50 μ L of Elution Buffer to the centre of the column and incubate at room temperature for 1 minute. Centrifuge for 1 minute to elute DNA. Ultrapure water (pH 7.5-8.5) may be used in place of elution buffer. However, DNA recovery with acidic waters may be significantly reduced.
11. Store the purified DNA at -20 °C.

Notes (elution step):

1. It is extremely important to add the Elution Buffer to the centre of the column. Incubating the column at higher temperatures (37 to 50 °C) may slightly increase the yield. Pre-warming the Elution Buffer at 55 to 80 °C may also slightly increase elution efficiency.
2. If a higher DNA concentration is desirable, 30 μ L (or less) of Elution Buffer can be used to elute the DNA. It is critical that the Elution Buffer is applied directly in the centre of the column. (To recover maximum amount of DNA it is recommended to repeat the elution step.)
3. If water is used for elution, make sure that its pH is between 7.5 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.

Protocol for PCR clean-up or DNA purification from enzymatic reactions

All purification steps should be carried out at **room temperature**.

All centrifugations should be carried out at **room temperature** in a table-top microcentrifuge at >12000 xg (10000-15000 rpm depending on the rotor type).

1. Transfer the volume of the reaction mixture into a 1.5 mL microcentrifuge tube and add five volumes of Binding Buffer. Mix by inverting the tube a few times. Centrifuge briefly to collect the sample. All purification steps including centrifugation should be carried out at **room temperature**.

Notes (sequencing of PCR products):

When cleaning up PCR products for subsequent sequencing processes, if using an amplification primer for sequencing avoid primers larger than 22-25 bp. In this case it is recommended to use a maximum of 0.25 μM of primers during the PCR amplification and 2.5 μM of the sequencing primer in the sequencing reaction. In case residual peaks appear in sequencing chromatograms, due to the presence of traces of the second amplification primer, dilute Binding Buffer to a 60-50% solution in water and proceed as above. Use Neo Biotech sequencing services for maximum efficiencies.

2. Add the above mixture to the Neo Biotech spin column. The maximum loading volume of the column is 700 μL . For sample volumes greater than 700 μL simply load again. Centrifuge for 30s to 1 minute and discard the flow-through in the tube.
3. Add 600 μL of Wash Buffer and centrifuge for 30s to 1 minute. Discard the flow-through in the collection tube.
4. Centrifuge for 1 minute to dry Neo Biotech spin membrane of residual ethanol.
5. Place the Neo Biotech spin column into a clean 1.5 mL microcentrifuge tube. Add 50 μL of Elution Buffer to the centre of the column and incubate at **room temperature** for 1 minute. Centrifuge for 1 minute to elute DNA. Ultrapure water may be used in place of elution buffer. However, DNA recovery with acidic waters may be significantly reduced. (Please check elution notes in previous section).
6. Store the purified DNA at $-20\text{ }^{\circ}\text{C}$.

Quality control assay

All components of Neo Biotech Gelpure kit are tested following the isolation protocol described above for the purification of DNA fragments from agarose gels and PCR reactions.