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NZYGelpure

Catalogue number MB01101

Presentation 50 columns

Description

NZYGelpure 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. NZYGelpure 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, enzymes, 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.

Shipping & Storage Conditions

This product is shipped at room temperature. All kit components can be stored at room temperature (15-25 °C) and are stable till the expiry date if stored as specified.

Components

COMPONENT	MB01101 (50 COLUMNS)
Binding Buffer	2 x 30 mL
Wash Buffer (concentrate)	12 mL
Elution Buffer (does not contain EDTA)	15 mL
NZYtech Spin Columns	50
Collection Tubes (2 mL)	50

Reagents, Materials and Equipment Required but Not Provided

- 96-100% ethanol
- 1,5 mL microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes

Standard Protocol

Procedures before starting

Reagents Preparation

- Wash Buffer: add 48 mL of 96-100% ethanol to the bottle of Wash Buffer.

Procedure for DNA Purification from Agarose Gels

All centrifugations should be carried out at **room temperature** in a table-top microcentrifuge at >12000 x *g* (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 NZYtech 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 µL of isopropanol).

6. Load the above mixture into the NZYtech spin column placed into a Collection tube (2 mL). Centrifuge for 30 s to 1 minute and discard the flow-through in the collection tube. The maximum volume of the column reservoir is 700 µL. For sample volumes of more than 700 µL, simply load and spin again.

7. (Optional) Add 500 µL of Wash Buffer and centrifuge for 30s to 1 minute. Discard the flow-through 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 µL 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 NZYtech spin membrane of residual ethanol.

10. Place the NZYtech 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 (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.

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

All centrifugations should be carried out at **room temperature** in a table-top microcentrifuge at >12000 x *g* (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 (for 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 NZYtech sequencing services for maximum efficiencies.*

2. Add the above mixture to the NZYtech 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 NZYtech spin membrane of residual ethanol.

5. Place the NZYech 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 °C.

Technical Notes

Elution procedures

- *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.*
- *If a higher DNA concentration is desirable, 30 µL 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).*

- *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.*

Quality control assay

All components of NZYGelpure kit are tested following the isolation protocol described above for the purification of DNA fragments.

Troubleshooting

Troubleshooting is often a systematic, meticulous process where varying one parameter at a time and evaluating impacts can unveil the root cause of issues. These adjusted suggestions, incorporating a blend of specificity and exploratory approaches, aim to enhance the clarity and actionability of your troubleshooting guide. Should any other technical or procedural aspects require attention, your feedback and additional information will always be welcomed.

LOW OR NO DNA YIELD
• **Gel band incompletely dissolved**
Ensure that the correct volume of Binding Buffer was added. Increase the incubation time of the mixture until the gel band is completely dissolved.
• **Inadequate Buffers preparation**
Check that Wash Buffer concentrated was diluted with correct volume of ethanol.
SUBOPTIMAL ELUTION
• **Low Elution Volume**
To improve elution efficiency, consider increasing the elution volume, with options of up to 100 µL. Alternatively, repeat the elution step up to three times.
LOW DNA QUALITY
• **Low A_{260}/A_{230} ratio**
Probably the eluate contains carry-over of chaotropic salts. Thiocyanate salts are effective and reliable for binding nucleic acids to a silica surface. Nevertheless, these chaotropic salts present high absorption at 230 nm affecting the A_{260}/A_{230} ratio. However, a low A_{260}/A_{230} not affect the quality of DNA, making a low A_{260}/A_{230} ratio insignificant for downstream applications. To improve the A_{260}/A_{230} ratio increase the number of washing steps with Wash Buffer.

By addressing these possible causes and implementing the suggested solutions, you can troubleshoot common issues encountered during the DNA isolation process and achieve better results with the NZYGelpure.