Gel Extraction / PCR and DNA Fragment Purification Kit

Item N°:

NB-03-0177 50 preps
NB-03-0178 100 preps
NB-03-0179 200 preps

Kit Content

Content	NB-03-0177	NB-03-0178	NB-03-0179
Solution BD	20 ml	40 ml	80 ml
Solution PE	15 ml	15 ml x2	20 ml x3
Eluent Buffer	2.5 ml	5 ml	10 ml
Spin column	50 each	100 each	200 each

Store

The kit can be stored for up to 12 months at room temperature (15-25°C) or at 4°C for storage periods longer than 12 months.
Any precipitate in the buffers can be re-dissolved by incubating at 37°C before use.

Description

This kit is designed to extract and purify DNA fragments of 50bp to 40kb from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE).This membrane-based system, which can bind up to 40µg DNA, allows recovery of isolated DNA fragments in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

This kit is also designed for rapid and efficient purification of DNA from PCR and other enzymatic reaction mixtures. The kit utilizes a proprietary silica-based membrane technology in the form of a convenient spin column, eliminating the need for tedious resin manipulations or toxic phenol-chloroform extractions. The PCR Purification Kit effectively removes primers, dNTPs, unincorporated labeled nucleotides, enzymes and salts from PCR and other reaction mixtures. The kit can be used for purification of DNA fragments from 50 bp to 40 kb

with recovery rates up to 100%. The purified DNA can be used in common downstream applications such as sequencing, restriction digestion, labeling, ligation, cloning, *in vitro* transcription, blotting or *in situ* hybridization.

Applications

Fast and efficient purification of DNA fragments ideal for use in all conventional molecular biology procedures including:

- Conventional restriction digestion
- Automated fluorescent or radioactive sequencing
- PCR
- *in vitro* transcription

GEL EXTRACTION

Principle

The dissolved gel piece is placed into a Quick Extraction Column containing a silica membrane. The DNA is bound to the membrane by either centrifugation or vacuum. The membrane is then washed with Wash Buffer containing ethanol to remove impurities and the purified DNA is then eluted into a Recovery Tube using Elution Buffer (10 mM Tris-HCl, pH 8.5). The purified DNA is suitable for use in a wide variety of downstream applications.

Important Notes

- Prior to the initial use of the kit, dilute the Wash Buffer(PE) with ethanol (100%) :

	NB-03-0177 (50preps)	NB-03-0178 (100preps)	NB-03-0179 (200preps)
Wash Buffer(PE)	15 ml	15 ml ×2	20 ml ×2
Ethanol	60 ml	60 ml ×2	80 ml ×2
Total Volume	75 ml	75 ml ×2	100 ml ×2

After the ethanol has been added, mark the check box on the bottle to indicate the completed step.

- Examine the Gel Solubilization Buffer (BD) for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.
- Freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running.
- All purification steps should be carried out at room temperature.

Procedure

1. Weigh a 1.5ml microcentrifuge tube for each DNA fragment to be isolated and record

the weight.

2. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 ml tube and weigh. Record the weight of the gel slice.

Note : If the purified fragment will be used for cloning reactions, avoid damaging the DNA through UV light exposure. Minimize UV exposure to a few seconds or keep the gel slice on a glass or plastic plate during UV illumination.

3. Add Gel Solubilization Buffer (BD) at a ratio of 10µl of solution per 10mg of agarose gel slices.

4. Incubate the gel mixture at 50-60°C for 10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved.

Note : Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is red, add 10 µl of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.

• High concentration gels (>2% agarose) or large gel slices may take longer than 10 minutes to dissolve.

5. Transfer the dissolved gel mixture to the Quick Gel Extraction Columns assembly and incubate for 2 minutes at room temperature.

6. Centrifuge the Quick Gel Extraction Columns assembly in a microcentrifuge at 14,000× g (12,000rpm) for 1 min, then discard the flow-through.

7. Wash the column by adding 500 µl of Wash Buffer (PE), to the Quick Gel Extraction Columns. Centrifuge the Quick Gel Extraction Columns assembly for 1 min at 14,000× g (12,000rpm), then discard the flow-through.

Note : Wash Buffer(PE) must be previously diluted with100% ethanol.

8. Wash the column by adding 500 µl of Wash Buffer (PE) again, previously diluted with 100% ethanol, to the Quick Gel Extraction Columns. Centrifuge the Quick Gel Extraction Columns assembly for 1 min at 14,000 × *g* (12,000rpm), then discard the flow-through.

9. Centrifuge the empty Quick Gel Extraction Columns for an additional 3 min to completely remove residual wash buffer.

10. Note. This step is essential to avoid residual ethanol in the purified DNA solution. The presence of ethanol in the DNA sample may inhibit downstream enzymatic reactions.

11. Empty the Collection Tube and recentrifuge the column assembly for 1 min with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

12. Carefully transfer the Quick Gel Extraction Columns to a clean 1.5ml microcentrifuge tube. Apply 50 µl of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly to the center of the column without touching the membrane with the pipette tip. Incubate at room

temperature for 2 minutes. Centrifuge for 1 min at 14,000× g (12,000rpm).

Note. For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µl does not significantly reduce the DNA yield. However, elution volumes less than 10 µl are not recommended.

• If DNA fragment is >10 kb, prewarm Elution Buffer to 65°C before applying to column.

• If the elution volume is 10 µl and DNA amount is >5 µg, incubate column for 1 minute at room temperature before centrifugation.

13. Discard the Quick Gel Extraction Columns and store the microcentrifuge tube containing the eluted DNA at 4°C or –20°C.

PCR AND DNA FRAGMENT PURIFICATION

Principle

A reaction mixture containing DNA is combined with the binding buffer and added to a purification column. A chaotropic agent in the binding buffer denatures proteins and promotes DNA binding to the silica membrane in the column. As an added convenience, the binding buffer contains a color indicator that allows for easy monitoring of the solution pH for optimal DNA binding. Impurities are removed with a simple wash step. Purified DNA is then eluted from the column with the Elution Buffer. The recovered DNA is ready for use in downstream applications.

Important Notes

• Prior to the initial use of the kit, dilute the Wash Buffer(PE) with ethanol (100%):

	NB-03-0177 (50preps)	NB-03-0178 (100preps)	NB-03-0179 (200preps)
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After the ethanol has been added, mark the check box on the bottle to indicate the completed step.

• Examine the Gel Solubilization Buffer (BD) for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.

• Freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running.

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

Protocol

1. Add a 1:1 volume of Solution BD to completed PCR mixture (e.g. for every 100 µl of reaction mixture, add 100 µl of Binding Buffer). Mix thoroughly.
2. Transfer up to 800 µl of the solution from step 1 to the spin column. Incubate for 2 minutes. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through.

***Note** : If the total volume exceeds 800 µl, the solution can be added to the column in stages. After the addition of 800 µl of solution, centrifuge the column for 30-60 s and discard flow-though. Repeat until the entire solution has been added to the column membrane.*

3. Add 500 µl of Solution PE (diluted with the ethanol) to the column. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through and place the purification column back into the collection tube. Repeat this step again.
4. Centrifuge the empty column for an additional 3 min to completely remove any residual wash buffer.

***Note** : This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.*

5. Transfer the column to a clean 1.5 ml microcentrifuge tube (not included). Add 30-100 µl of Elution Buffer (prewarm to 65℃)to the center of the column membrane and incubate for 2 minutes. Centrifuge for 1 min at 12,000 rpm. Discard the column and store the purified DNA at -20℃.

***Note :** For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µl does not significantly reduce the DNA yield. However, elution volumes less than 10 µl are not recommended.*

Quality Control

The kit is tested in the purification of 50 bp and 1 kb PCR products according to the protocol. The quality of the purified DNA is evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with restriction enzymes and automated fluorescent sequencing.