MEGAquick-spin™ Plus Total Fragment DNA Purification Kit

REF 17290 | 15℃ 25℃ | RUO | Σ 200

DESCRIPTION

MEGAquick-spin™ Plus Total Fragment DNA Purification Kit is designed to extract and purify DNA fragments of 65 bp ~ 20kb from normal or low melt agarose gels, or to purify PCR products directly from a PCR amplification and DNA cleanup from other enzymatic reactions.

Recovery is achieved up to 98% incase of PCR cleanup. PCR products are commonly purified to remove excess nucleotides and primers. This membrane-based system, which can bind up to 20 µg DNA, allows recovery of isolated DNA fragments or PCR products in as little as 20 minutes. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription / translation without further manipulation.

SPECIFICATION

- Principle : spin column (silica membrane)
- DNA binding capacity of spin column : 20 µg
- Sample size : up to 300 mg of agarose gel / up to 100 µl of reaction solution
- DNA size : 65 bp ~ 20 kb
- Recovery : 90 ~ 98% for PCR purification
- Operation time : PCR purification : 10 / Gel extraction : 20 min
- Elution volume : 40 µl

KIT CONTENTS

No.	Contents	Unit
1	BNL Buffer[1]	160 ml x 1 bottle
2	Washing Buffer[2]	45 ml x 1 bottle
3	Elution Buffer	20 ml x 1 bottle
4	Spin Column / Collection Tube	200 ea

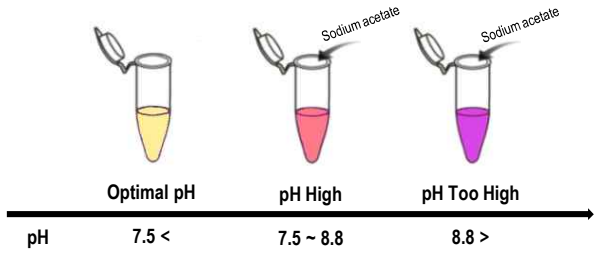
[1] BNL Buffer contains chaotropic salts which are irritants. Take appropriate laboratory safety measures and ware gloves when handling.

[2] Washing Buffer is supplied as concentrate. Add 180 ml per bottle of absolute ethanol according to the bottle label before use.

STORAGE CONDITION

- MEGAquick-spin™ Plus Total Fragment DNA Purification Kit should be stored at room temperature.
- It can be stored for up to 24 months without showing any reduction in performance and quality.

Effect of pH on DNA Binding



BNL Buffer contains an integrated pH indicator allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires a pH 7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5, which can occur if during agarose gel electrophoresis, the electrophoresis buffer had been used repeatedly or incorrectly prepared, or if the buffer used in an enzymatic reaction is strongly basic and has a high buffering capacity, the binding mixture turns orange or violet. This means that the pH of the sample exceeds the buffering capacity of BNL Buffer and DNA adsorption will be inefficient. In these cases, the pH of the binding mixture can easily be corrected by addition of a small volume of 3 M sodium acetate, pH 5.0, before proceeding with the protocol.

ADDITIONAL REQUIRED EQUIMENT

- Absolute ethanol
- sterilized microcentrifuge tubes (1.5 ml)
- Standard tabletop micro-centrifuge
- Heat block or Water bath (for Gel extraction)
- Agarose; scalpel

PROTOCOLS

◆ PCR Purification

1. **Transfer up to 100 µl of PCR product (excluding oil) to a microcentrifuge tube (not provided) and add 5 volumes of BNL Buffer, mix well by vortexing.**
   Note : For example, Add 250 µl of BNL Buffer to 50 µl of PCR product.
   Note : The maximum volume of PCR product is 100 µl (excluding oil). Do not excess this limit. If PCR product is more than 100 µl, separate it into multiple tubes.
2. **Prepare a Spin column.**
3. **Transfer the sample mixture to the Column. Centrifuge at 18,000 x g (13,000 rpm) for 30 seconds, then discard the flow-through.**
4. **Add 750 µl of Washing Buffer (ethanol added) to the Column. Centrifuge at 18,000 x g (13,000 rpm) for 30 seconds, then discard the flow-through.**
   Note : Make sure that absolute ethanol has been added into Washing Buffer when first open.
5. **Centrifuge again at full speed ~18,000 x g (13,000 rpm) for an additional 3 minutes to dry the column membrane.**
   Note : The residual liquid should be removed thoroughly on this step.
6. **Place the Column to a new microcentrifuge tube (not provided).**
7. **Add 40 µl of Elution Buffer to the membrane center of the Column. Stand the Column for 1 min.**
   Note : For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely
   Note : Do not elute the DNA using less than suggested volume (40 µl). It will lower the final yield.
8. **Centrifuge at full speed ~18,000 x g (13,000 rpm) for 1 min to elute the DNA.**

◆ Gel Extraction

* Prepare heat block or water bath at 55℃ before starting.

1. **Excise the agarose gel with a clean scalpel.**
   Note : Remove the extra agarose gel to minimize the size of the gel slice.
2. **Transfer up to 300 mg of the gel slice into a microcentrifuge tube. (not provided).**
   Note : The maximum volume of the gel slice is 300 mg.
3. **Add 500 µl of BNL Buffer to the sample and mix by vortexing.**
   Note : For > 2% agarose gels, add 1000 µl of BNL Buffer.
4. **Incubate at 55℃ for 5 ~ 10 minutes and vortex the tube every 2 ~ 3 minutes until the gel slice dissolved completely.**
   Note : During incubation, periodic vortexing can accelerate dissolving of gel.
   Note : Make sure that the gel slice has been dissolved completely before proceed the next step.
   Note : After gel dissolved, make sure that the color of sample mixture is yellow. If the color is violet, add 10 µl of sodium acetate, 3M, pH 5.0. Mix well to make the color of sample mixture turned to yellow.
5. **Cool down the sample mixture to room temperature. And place a column into a collection tube.**
6. **Transfer 800 µl of the sample mixture to the column. Centrifuge at 18,000 x g (13,000 rpm) for 30 seconds, then discard the flow-through.**
   Note : If the sample mixture is more than 800 µl, repeat this step for the rest of the sample mixture.
7. **Add 750 µl of Washing Buffer (ethanol added) to the column. Centrifuge at 18,000 x g (13,000 rpm) for 30 seconds, then discard the flow-through.**
   Note : Make sure that absolute ethanol has been added into washing buffer when first use.
8. **Centrifuge again at full speed ~ 18,000 x g (13,000 rpm) for an additional 2 minutes to dry the column membrane.**
   Note : The residual liquid should be removed thoroughly on this step.
9. **Place the column to a new microcentrifuge tube (not provided).**

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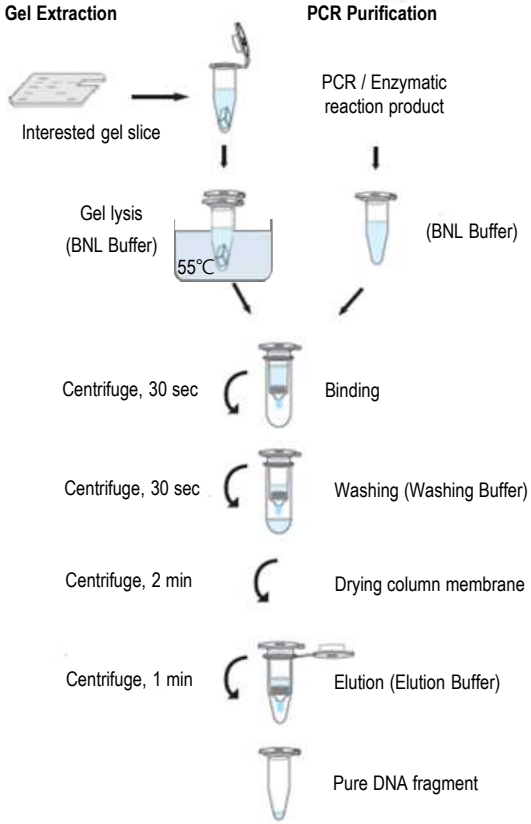
10. **Add 40 µl of elution buffer to the membrane center of the column. Stand the column for 1 min.**
    Note : For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
    Note : Do not elute the DNA using less than suggested volume (40 µl). It will lower the final yield.
11. **Centrifuge at full speed ~ 18,000 x g (13,000 rpm) for 1 min to elute the DNA.**

BRIEF PROTOCOL



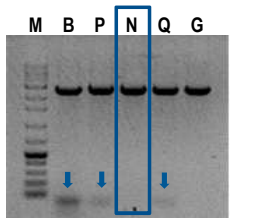
* All centrifuge steps run at 18,000 x g (13,000 rpm)

TROUBLE SHOOTING GUIDE

Problem	Possible causes and comments
Low or none recovery of DNA fragment	**Apply more than 100 µl of PCR product** • If PCR product is more than 100 µl, separate it into multiple tubes.
	The column is loaded with too much agarose gel • The maximum volume of the gel slice is 300 mg per column.
	Elution of DNA fragment is not efficient • Make sure the pH of Elution Buffer or ddH_2O is between 7.0 ~ 8.5. • Make sure that the elution solution has been completely absorbed by the column membrane before centrifugation.
	The size of DNA fragment is smaller than 200 bp • Add 1 volume of isopropanol before binding step.
	The size of DNA fragment is larger than 5 kb • Preheat the elution solution to 60℃ before centrifugation.
The gel slice is hard to dissolve	**Agarose gel of high percentage (>2%) is used** • Add 1000 µl of BNL Buffer to 1 volume of the gel slice
	The size of the gel slice is too large • If the gel slice is more than 300 mg, separate it into multiple tubes.
Eluted DNA contains non-specific DNA fragment	**Contaminated scalpel** • Using a new or clean scalpel.
	DNA fragment is denatured • Incubate eluted DNA at 95℃ for 2 min, then cool down slowly to reanneal denatured DNA.

TECHNICAL INFORMATION

※ Comparative test with Other company (PCR Purification)



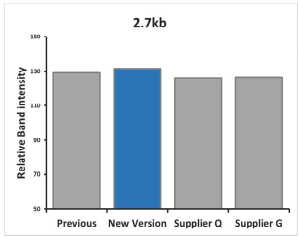
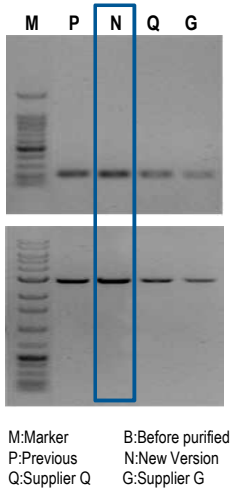


Fig.1. PCR Purification.
As a result of electrophoresis for PCR product (2.7 kb) purification, the recovery rate was higher than that of other products, and the primer dimer was effectively removed.

※ Comparative test with Other company (Gel Extraction)



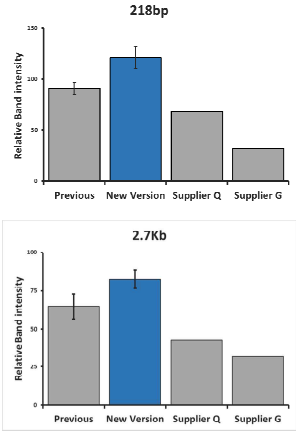
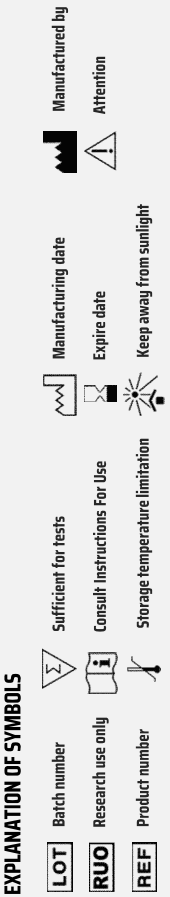


Fig.2. Gel Extraction.
As a result of the electrophoresis for the purification of the PCR product (218 bp, 2.7 kb), it was confirmed that the recovery rate was higher than that of other products.

ORDERING INFORMATION

Product Name	Amout	Cat. No
SiZer™-DNA Marker Solution Series	0.5 ml	24073 / 24074
Patho Gene-spin™ DNA/RNA Extraction Kit	50 col.	17154
Fast DNA-spin™ Plasmid DNA Purification Kit	200 col.	17013
2 x PCR Master mix solution (i-pfu)	0.5ml x 2 vials	25186
i-pfu DNA polymerase	250 Units	25181
Maxime™ PCR PreMix (i-pfu)	96 tubes	25185
Maxime™ PCR PreMix (i-Taq)	96 tubes	25025
Maxime™ PCR PreMix (i-StarTaq)	96 / 480 tubes	25165 / 25167
Maxime™ PCR PreMix (i-MAX Ⅱ)	96 tubes	25265

EXPLANATION OF SYMBOLS



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