MEGAquick-spin™ Plus Total Fragment DNA Purification Kit

RUO *Research Use Only* REF *17290* Σ 200 15℃ 25℃

PRODUCT FEATURES

- For extraction of DNA fragments from agarose gel
- For purification of PCR products or reaction mixtures(concentration and desalination of reaction mixtures)

INTRODUCTION

The MEGAquick-spin™ Plus Total Fragment DNA Purification Kit is designed to extract and purify DNA fragments of 65 bp ~ 10 kb from normal or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE), or to purify PCR products directly from a PCR amplification and DNA cleanup from other enzymatic reactions. Recovery is achieved up to 95% in case 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, 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.

KIT CONTENTS

Components	200 prep
BNL Buffer / Plus (Agarose gel lysis buffer)[1]	160 ml
Washing Buffer / Plus[2]	45 ml
Elution Buffer / Plus	20 ml
Spin Column	200 ea
Collection Tube	200 ea
Storage Conditions : Room Temperature	

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

[2] Washing Buffer / Plus is supplied as concentrate. Add 180 ml per bottle of ethanol (96~100%) according to the bottle label before use.

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 ~ 10 kb
- Recovery : 70 ~ 85% for Gel extraction / 90 ~ 95% for PCR clean up
- Operation time : 10 ~ 20 min
- Elution volume : 40 μl

INPORTANT NOTES

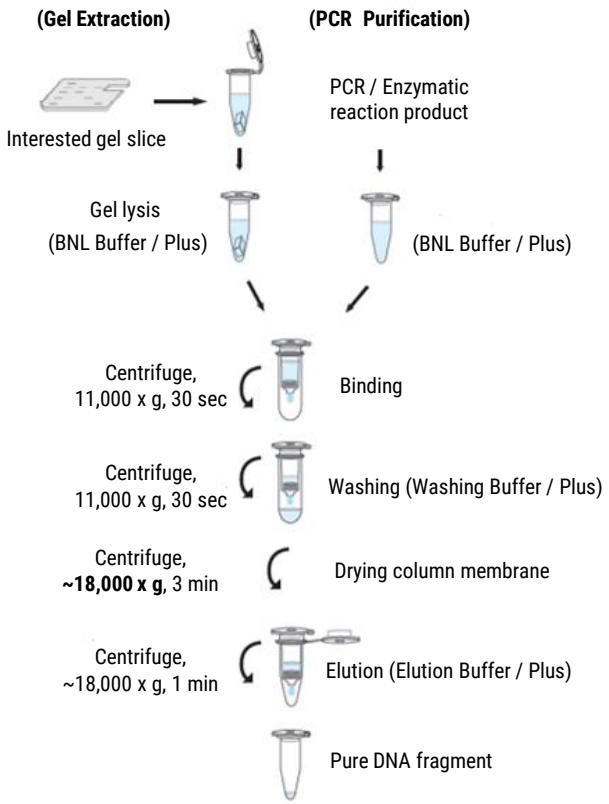
1. Buffer provided in this kit contain irritants. Wear gloves and lab coat when handling these buffer.
2. Add the required volume of ethanol (96 ~100%) to Washing Buffer / Plus before use.
3. Centrifugation steps are done by a microcentrifuge capable of the speed at 11,000 ~ 18,000 x g.

Hint : Prepare a 55℃ dry bath ro water bath for step 4.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. iNtRON is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the **MEGAquick-spin™ Plus Total Fragment DNA Purification Kit**, please do not hesitate to contact us. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. For technical assistance and more information please call iNtRON's local distributors.

BRIEF PROCEDURE



PROTOCOL : Gel Extraction

1. Excise the agarose gel with a clean scalpel.
   - 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).
   - The maximum volume of the gel slice is 300 mg.
3. Add 500 μl of BNL Buffer / Plus to the sample and mix by vortexing.
   - For > 2% agarose gels, add 1000 μl of BNL Buffer / Plus.
4. Incubate at 55℃ for 5 ~ 10 minutes and vortex the tube every 2 ~ 3 minutes until the gel slice dissolved completely.
   - During incubation, interval vortexing can accelerate the gel dissolved.
   - Make sure that the gel slice has been dissolved completely before proceed the next step.
   - 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 11,000 x g for 30 seconds, then discard the flow-through.
   - 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 / Plus (ethanol added) to the Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.
   - Make sure that ethanol (96 – 100%) has been added into Washing Buffer / Plus when first use.

8. Centrifuge again at full speed (~ 18,000 x g) for an additional 3 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).
10. Add 40 µl of Elution Buffer or ddH_20 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) for 1 min to elute the DNA.

PROTOCOL : 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 / Plus, mix well by vortexing.
   - For example, Add 250 µl of BNL Buffer / Plus to 50 µl of PCR product.
   - 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. Place a Column into a Collection Tube.
3. Transfer the sample mixture to the Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.
4. Add 750 µl of Washing Buffer / Plus (ethanol added) to the Column. Centrifuge at 11,000 x g for 30 seconds, then discard the flow-through.
   - Make sure that ethanol (96-100%) has been added into Washing Buffer / Plus when first open.
5. Centrifuge again at full speed (~18,000 x g) 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 / Plus or ddH_2O 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) for 1 min to elute the DNA.

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
Low or none recovery of DNA fragment	
1)Apply more than 100 µl of PCR product	• If PCR product is more than 100 µl, separate it into multiple tubes.
2)The column is loaded with too much agarose gel	• The maximum volume of the gel slice is 300 mg per column.
3)Elution of DNA fragment is not efficient	• Make sure the pH of Elution Buffer / Plus 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.
4)The size of DNA fragment is larger than 5kb	• Preheat the elution solution to 60℃ before centrifugation.
The gel slice is hard to dissolve	
1)Agarose gel of high percentage (>2%) is used	• Add 1000 µl of BNL Buffer / Plus to 1 volume of the gel slice.
2)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	
1)Contaminated scalpel	• Using a new or clean scalpel.
2)DNA fragment is denatured	• Incubate eluted DNA at 95℃ for 2 min, then cool down slowly to reanneal denatured DNA.
Poor performance in the downstream applications	
1)Salt residue remains in eluted DNA 2)Ethanol residue remains in eluted DNA	• Wash the column twice with Washing Buffer / Plus. • Do discard the flow-through after washing with Washing Buffer / Plus and centrifuge for an additional 3 min.

ORDERING INFORMATION

Product Name	Amount	Cat. No.
SiZer™-100 plus DNA Marker Solution	0.5 ml	24078
Fast DNA-spin™ Plasmid DNA Purification Kit	50/200 col.	17095/17013
DNA-midi™ GT Plasmid DNA Purification Kit	25 col.	17254
Patho Gene-spin™ DNA/RNA Extraction Kit	50 col.	17154
Fast HQ RNA Extraction Kit	50 col.	17213
MacCell™ DH5α 10^7 / 10^8 / 10^9	1 ml	15052/15053/15054
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

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