

WizPrep™ Gel/PCR Purification Mini Kit

RUO For Research Use Only

REF W70150

DESCRIPTION

The WizPrep™ Gel/PCR Purification Mini Kit was designed to recover or concentrate DNA fragments (100 bp→10 Kb) from agarose gel, PCR, or other enzymatic reactions. Chaotropic salt is used to dissolve agarose gel and denature enzymes. DNA fragments in the chaotropic salt are bound by the glass fiber matrix of the spin column.

The unique dual purpose application and high yield DNA/ minicolumn make this kit an exceptional value. The method uses a chaotropic salt, guanidine thiocyanate to dissolve agarose gel and denature enzymes. DNA fragments in chaotropic salt solution bind to the glass fiber matrix of the spin column. Following washing off of contaminants, the purified DNA fragments are eluted by addition of low salt elution buffer or water. Salts, enzymes and unincorporated nucleotides are effectively removed from reaction mixtures without phenol extraction or alcohol precipitation.

KIT CONTENTS

| Contents | 100 prep | 300 prep | Storage |
|--|----------|----------|------------|
| GP Buffer | 60 ml | 180 ml | Room temp. |
| Wash Buffer (concentrate) ⁽¹⁾ | 16 ml | 50 ml | Room temp. |
| Elution Buffer | 10 ml | 30 ml | Room temp. |
| Spin Columns* | 100 | 300 | Room temp. |
| Instruction Manual | 1 | 1 | |

(1) : Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

* All Spin Columns are sterilized by electron beam.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY USER

- 96-100% ethanol (to prepare Wash Buffer)
- 1.5 mL microcentrifuge tubes
- Sterile RNase-free pipette tips
- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

KIT SPECIFICATIONS

- Fast and easy processing using a rapid spin-column format
- High column binding capacity – up to 25 µg
- Sample: up to 300 mg of agarose gel
up to 20 µl of reaction solution
- Recovery: 70% - 85% for Gel extraction
90% - 95% for PCR clean-up
- Operation time: 20 min
- Elution volume: 50 µl

QUALITY CONTROL ANALYSIS

In accordance with Wizbiosolutions Inc. ISO 13485-certified Quality Management System, each lot WizPrep™ Gel/PCR Purification Mini Kit is tested against predetermined specifications to ensure consistent product quality.

PROTOCOL

To check before start

- Before starting, add 64(200)ml of 100% ethanol to the 16(50)ml of Wash Buffer concentrate.

Gel Extraction

- 1) Excise the DNA band of interest as small and rapid as possible.
- 2) Add **3 volume (µl) of GP Buffer** to **1 volume (mg) of gel**.
- 3) Incubate at 50°C with occasional mixing until the gel is completely melted.
Note: Check that the color of mixture is yellow after melting. If the color change to orange or violet, addition of a small volume of **3M sodium acetate**.
- 4) (Optional) Add **1 gel volume of isopropanol** to the sample and vortex to mix. This step is required to increase the recover yields of DNA fragments <200 bp or >5 kb.
- 5) Transfer the mixture to a Spin Column
- 6) Centrifuge at 13,000 rpm for 1 min. and discard the filtrate.
- 7) Add **700µl of Wash Buffer** (ethanol added) to the Spin Column
- 8) Centrifuge at 13,000 rpm for 30 sec. and discard the filtrate.
- 9) Centrifuge for an additional 1 min. and transfer the Spin Column to a new 1.5 ml tube.
- 10) Apply **50µl of Elution Buffer** into the center of the column matrix.
- 11) Let stand for 1 min.
- 12) Centrifuge at 13,000 rpm for 1 min.

PCR Clean-up

- 1) Transfer the PCR product to 1.5ml tube.
- 2) Add **5 volume of GP Buffer** to 1 volume of the sample and mix thoroughly.
- 3) Transfer the mixture to a Spin Column
- 4) Centrifuge at 13,000 rpm for 1 min. and discard the filtrate.
- 5) Add **700µl of Wash Buffer** (ethanol added) to the Spin Column
- 6) Centrifuge at 13,000 rpm for 30 sec. and discard the filtrate.
- 7) Centrifuge for an additional 1 min. and transfer the Spin Column to a new 1.5 ml tube.
- 8) Apply **50µl of Elution Buffer** into the center of the column matrix.
Note: For larger fragment (>5 kb), use pre-warmed (70°C) elution buffer for best efficiency.
- 9) Let stand for 1 min.
- 10) Centrifuge at 13,000 rpm for 1 min.

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TROUBLESHOOTING

| Problem | Possibility | Suggestions |
|---|---|--|
| Low yield of Gel/PCR Purification | Agarose gel did not dissolve completely. | <ul style="list-style-type: none">• Ensure the agarose gel was dissolved completely between 55-60°C for 10-15 minutes, or until no gel is visible.• If undissolved agarose remains in the sample, the Column could clog and some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60°C.• If using more than 300 mg of agarose gel, separate it into multiple 1.5 ml microcentrifuge tubes. |
| | Incomplete Wash Buffer preparation. | <ul style="list-style-type: none">• Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds.• Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. |
| | Incorrect DNA elution step. | <ul style="list-style-type: none">• Ensure that Elution Buffer is added into the center of the Column matrix and is completely absorbed.• If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer (60-70°C). |
| Eluted DNA does not perform well in downstream applications | <p>DNA was denatured (a smaller band appeared on gel analysis).</p> <p>Primer dimer contamination in the final PCR elution product.</p> | <ul style="list-style-type: none">• Ensure the agarose gel was dissolved completely between 55-60°C for 10-15 minutes, or until no gel is visible.• DNA can be denatured if the incubation temperature exceeds 60°C.• Incubate the eluted DNA at 95°C for 2 minutes then cool down slowly to re-anneal the denatured DNA.• Gel purification should be performed if primer dimers are visible in the agarose gel following PCR reactions.• Simply cut the PCR product from the gel and avoid the primer dimer. Using an additional 80% ethanol wash will reduce primer dimer contamination when performing PCR cleanup. |

SYMBOL GLOSSARY

| | | | |
|---|-------------------|---|----------------------|
| REF | Catalogue number |  | Manufacturer |
| LOT | Batch code | RUO | Research use only |
|  | Temperature limit |  | Instructions for use |
|  | Use-by date | | |

ORDERING INFORMATION

| Product | Cat No. | Package |
|--|------------|----------|
| WizPrep™ Gel/PCR Purification Mini Kit | W70150-100 | 100 prep |
| | W70150-300 | 300 Prep |



Wizbiosolutions Inc.

#1103, 1405, 1406, A-dong, 14, Sagimakgol-ro 45beon-gil,
Jungwon-gu, Seongnam-si, Gyeonggi-do, Republic of Korea
B237~B242, 14, Galmachi-ro 288beon-gil, Jungwon-gu,
Seongnam-si, Gyeonggi-do, Republic of Korea

Tel : (82) 70-7603-5066

Fax: (82) 31-624-3066

e-mail: support@wizbiosolution.com

www.wizbiosolution.com

