Protech Technology

Gene-Spin™ 1-4-3 DNA Purification Kit - V^2

(For PCR/DNA clean-up & Gel Extraction)

Innovative Tools for Nucleic Acid Purification

This Kit is for research purposes only.
Not for use in diagnostic procedures.
For in vitro use only

Please do not hesitate to contact us if you have any questions.
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KIT CONTENTS :

Gene-Spin 1-4-3 kit	50 reactions	250 reactions
Cat No.	DNA143-V2	DNA143XL-V2
Binding Buffer	55 ml	275 ml
Washing Buffer	**16 ml** *1	**80 ml** *1
Elution Solution	10 ml	50 ml
Gene-Spin spin Column	50 pcs	250 pcs
Collection tubes	50 pcs	250 pcs

REMARKS BEFORE YOU START :

1. Before Using Washing Buffer, add 64 ml (for 50 reactions) or 320 ml (for 250 reactions) 95~100 % ethanol and mix well.

ATTENTION :

This protocol is designed to purify PCR product from primers, primer dimers, nucleotides, polymerase and salts. It only can remove DNA fragment < 70 bp, for completely removing DNA between 70~100 bp, please use gel purification protocol (see next page).

DESCRIPTION :

The Gene-Spin™ 1-4-3 DNA Purification Kit is designed to extrat and purify DNA fragments of 100 bp to 10 kb from TAE/TBE agarose gels, or purify PCR products directly from a PCR reaction. This membrane-based system, which can bind up to 40 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 fragments can be used directly for automated fluorescent DNA Sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/ translation without further manipulation.

THE Gene-Spin BASIC PRINCIPLE

The **Gene-Spin 1-4-3 DNA purification** method, DNA binding in the presence of chaotropic salts (binding buffer) to a silica membrane. Binding buffer contains additional components in order to dissolve agarose gel slices. Afterwards, DNA mixtures are loaded directly onto **Gene-spin columns**. Contaminations like salts and soluble macromolecular components are removed by a simple washing step with ethanolic binding buffer. Pure DNA is finally eluted under low ionic strength conditions with slightly buffer or water.

The procedure consist of three basic steps:

- Preparation and dissolving agarose gel slices
- Binding of DNA fragments onto the Gene-Spin Column
- Washing and elution of DNA fragments

Gene-Spin™ 1-4-3 Extraction for

DNA / PCR Clean-up Flow Chart

1 vol sample + 5 vol Binding Buffer

Mix complete

Load DNA Mixtures ~ 700 ul

12,000 ~ 14, 000 x g , 1 min

+ 700 ul Washing Buffer

12,000 ~ 14, 000 x g , 1 min

Discard the filtrate

12,000 ~ 14, 000 x g , 3 min

Dry the spin column

Incubate 37~60^{0}C , 5 min

+ 30~50 ul Elution Buffer / ddH_2O

12,000 ~ 14, 000 x g , 1 min

GENERAL PROTOCOL FRO DNA AND PCR CLEAN-UP:

1. For each PCR solution or other DNA solution (after enzymatic treatment), transfer the solution to clear microcentrifuge tube.

2. For PCR & DNA solution volume exceeds 100 ul, add 5 volumes of **Binding Buffer** (e.g. 100 ul PCR reaction mix and **500 ul Binding Buffer**).

Tech. Note:

1. For PCR & DNA solution < 100 ul adjust the volume of solution mix to 100 ul using TE buffer (pH 7.5).

2. The maximum of volume of the column is 750 ul, if sample volume more than 750 ul, just load the remaining again after first spin in step 3, repeat step 3 & 4.

3. Insert the **spin column** into **collection tube**. Transfer the solution to **spin column** and spin for **1 min** at maximum speed (12,000~14,000 x g).

4. Remove the **spin column** from the **collection tube**, discard the filtrate then add **700 ul Washing Buffer** and spin for **1 min** at maximum speed (12,000~14,000 x g).

Tech. Note: For fluorescent sequencing, additional wash is recommended**.**

5. Discard the filtrate then centrifuge for **3 min** at top speed to remove residual trace of ethanol, additionally incubate the **spin column** at 37~60^{0}C oven for **5 min** to evaporate all the ethanol before elution**. (Do not incubate for longer than 10 min)**

Tech. Note: Residual ethanol from **Washing Buffer** would inhibit subsequent reactions. This step is very important for fluorescent sequencing and oven incubation will increase DNA yield.

6. Place the **spin column** into a clean microcentrifuge tube. To elute DNA, add **30~50 ul Elution Buffer** or H_2O (pH > 7.0) or TE buffer into the column, incubate at room temperature for **1 min** to increase the yield of eluted DNA. Spin for **1 min** at maximum speed (12,000 ~14,000 x g).

Tech. Note:

1. For DNA larger fragments (5~10 kb), using preheated 60~70^{0}C **Elution Buffer** or H_2O or TE to elute.
2. When using water to elute, make sure the pH value is within 7.0~8.5. Lower pH may cause lower DNA recovery.
3. For florescent sequencing, use only H_2O to elute DNA.

Gene-Spin™ 1-4-3 Extraction for

Gel Extraction Flow Chart

Excise DNA fragment

100 mg + 100 ul Binding Buffer

Dissolve agarose gel at 60^{0}C , 5~15 min

Load DNA Mixtures ~ 700 ul

12,000 ~ 14, 000 x g , 1 min

+ 500 ul Binding Buffer

12,000 ~ 14, 000 x g , 1 min

+ 700 ul Washing Buffer

12,000 ~ 14, 000 x g , 1 min

Discard the filtrate

12,000 ~ 14, 000 x g , 3 min

Dry the spin column

Incubate 37~60^{0}C , 5 min

+ 30~50 ul Elution Buffer / ddH_2O

12,000 ~ 14, 000 x g , 1 min

GENERAL PROTOCOL FOR GEL EXTRACTION:

1. Excise the desired DNA band (≤ 350 mg) using a clean scalpel, cut the slice into several small pieces and weight the gel in sterile microcentrifuge tube.

2. For each **100 mg** agarose gel add **100 ul Binding Buffer**. Incubate sample at 60^{0}C until the gel slices are completely dissolved (5~15 min). Vortex the sample briefly every 2~3 min until the gel slices are dissolved completely.

Tech. Note:

1. For > 2% gel, add 2~3 volumes Binding Buffer.

2. Add more Binding buffer to speed-up the melting time, but it will not increase the DNA recovery.

3. Insert the **spin column** into **collection tube**. Transfer the dissolved solution to **spin column** and spin for **1 min** at maximum speed (12,000~14,000 x g), remove the **spin column** from the **collection tube**, discard the filtrate.

4. Optional: Add **500 ul Binding Buffer** and spin for **1 min** at maximum speed (12,000~14,000 x g), remove the **spin column** from the **collection tube**, discard the filtrate.

Tech. Note: This step will remove any residual agarose, which might inhibit the enzymatic reaction of purified DNA. It is recommended for agarose gel >2 % and DNA for fluorescent sequencing.

5. Add **700 ul Washing Buffer** and spin for **1 min** at maximum speed (12,000~14,000 x g).

Tech. Note: For fluorescent sequencing, additional wash is recommended.

6. Discard the filtrate then centrifuge for **3 min** at top speed to remove residual trace of ethanol, additionally incubate the **spin column** at 37~60^{0}C oven for **5 min** to evaporate all the ethanol before eluting the DNA**. (Do not incubate for longer than 10 min)**

Tech. Note: Residual ethanol from **Washing Buffer** would inhibit subsequent reactions. This step is very important for fluorescent sequencing and oven incubation will increase DNA yield.

7. Place the **spin column** into a clean microcentrifuge tube. To elute DNA, add **30~50 ul Elution Buffer** or H_2O (pH > 7.0) or TE buffer into the column and spin for **1 min** at maximum speed (12,000~ 14,000 x g).

Tech. Note:

1. For DNA larger fragments (5~10 kb), using preheated 60~70^{0}C **Elution Buffer** or H_2O or TE to elute. Incubation on the silica membrane at room temperature for 3 min before centrifuge.

2. When using water to elute, make sure the pH value is within 7.0~8.5. Lower pH may cause lower DNA recovery.

3. For florescent sequencing, use only H_2O to elute DNA.

4. If checking DNA on agarose gel after elution in H_2O, add 1 volumes of **Elution Buffer** in DNA/loading dye mixture, or DNA may run faster than expected position.

TROUBLESHOOTING

Q: Incomplete lysis of agarose slices

⇨ *High concentration of agaeose* - Use doubled volumes of **Binding Buffer** for highly concentrated and/or LMP (low melting point) agarose gels.

⇨ *Time and temperature* - Check incubation temperature. Depending on the weight of gel slice, incubation (step 2) can be prolonged up to 20 min. Vortex every 2 min and check integrity of the gel slice. Heavy weight gel slices may be quenched or crushed before addition of **Binding Buffer**.

Q: No DNA Yield

⇨ *Reagents not applied properly* - Add indicated volume of 95~100% ethanol to **Washing Buffer** concentrate and mix well before use.

⇨ *Insufficient drying of the **Gene-Spin** silica membrane* - Centrifuge 3 min at 12,000~14,000 x g before elution to remove residual trace of ethanol completely. Additionally incubate the **spin column** at 37~60^{0}C oven for 5 min to evaporate all the ethanol before eluting the DNA**. (Do not incubate for longer than 10 min)**

⇨ *Isolation of large DNA fragments* - For DNA larger fragments (5~10 kb), using preheated 60~70^{0}C **Elution Buffer** H_2O or TE to elute. Incubation on the silica membrane at room temperature for 3 min before centrifuge.

Q: Poor results with automated fluorescent sequencing

⇨ *TE buffer used for DNA elution* - Ethanol precippitate the DNA or repurify the DNA fragments and elute with nuclease-free water.

⇨ *Too little DNA added to the sequencing reaction* - Increase the amounts of DNA used in the sequencing reaction or concentrate the DNA using ethanol precipitation. Up to 7 ul of the eluted DNA can be used per fluorescent sequencing reaction.

⇨ *Too much DNA added to the sequencing reaction* - Too much DNA can interfere with fluorescent sequencing. Use less eluted DNA or dilute DNA prior to sequencing.

Q: Poor restriction digestion

⇨ *Ethanol or salt carryover into the eluted DNA* - Ethanol precipitate the DNA or keep the volume of DNA to 10% or less of the final reaction volume.

Q: Low A_{260}/A_{230} ratios

⇨ *Typically due to guanidine isothiocyanate contamination* - Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. Ethanol precipitate the DNA if a low A_{260}/A_{230} ratio is a concern.

Q: Purified DNA floats out of the well when loaded on a gel

⇨ *DNA sheared* - Mix the agarose gel slice gently with the Binding Buffer.

⇨ *DNA degraded by nucleases* - Autoclave the gel running buffer before use. Store the gel slice at 4^0C or at -20^0C for no more than 1 week under nuclease-free conditions.

Q: Low cloning efficiency

⇨ *Likely due to guanidine isothiocyanate contamination* - Ethanol procipitate the DNA, washing the pellet with 70% ethanol, to reduce contamination.

Manufactured for and distributed by
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