Protech Technology

Gene-Spin™
PCR Clean-up
& Gel Extraction Kit

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

Innovative Tools for Nucleic Acid Purification

This kit is for Research Use Only (RUO).
Not for diagnostic or any other human in vivo procedures

Please feel free to contact us while you have any questions.
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Kit Contents

Reaction	50 rxns	250 rxns	Sample (6 rxns)
Cat No.	CG050	CG250	SA-CG006
Binding Buffer	55 ml	140 ml x 2	1.2 ml x 3
Washing Buffer	**16 ml** *1	**40 ml x 2** *1	**3.2 ml** *1
Elution Solution	10 ml	50 ml	1.2 ml
Gene-Spin™ Column	50 pcs	250 pcs	6 pcs
Collection Tube	50 pcs	250 pcs	6 pcs

Remarks Before You Start :

*1. Before using Washing Buffer, please add 64 ml (for 50 reactions) or 160 ml (for 250 reactions) or 12.8 mL (for sample) 95~100% ethanol and mix well.

Attention :

DNA/PCR Clean-up protocol is designed to remove DNA fragment < 70 bp, primers, nucleotides, polymerase and salts from DNA/PCR products. For completely removing DNA between 70~100 bp, please use Gel Extraction protocol (see page 8).

Product Description

The Gene-Spin™ PCR Clean-up & Gel Extraction Kit is designed to extract and purify DNA fragments of 100 bp to 10 kb from TAE/TBE agarose gels, or purify PCR products directly from a PCR reaction mix.

Binding capacity of Gene-Spin™ Column is up to 25 µg DNA. The entire procedure can be complete within 20 minutes. (The time spent is 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.

Basic Principle

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™ Column**. Contaminations like salts and soluble macromolecular components are removed by a simple wash step with Washing Buffer containing ethanol. After that, pure DNA is eluted under low ionic strength conditions with Elution Buffer, waters, or other suitable buffers.

The procedure consist of three basic steps:

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

Flow Chart of PCR Clean-up

1 vol sample + 5 vol Binding Buffer

Mix completely

Loading DNA mixtures (~ 700 µl) on Gene-Spin™ Column

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

+ 700 µl Washing Buffer

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

Discard the filtrate

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

Dry the Gene-Spin™ Column

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

+ 30~50 µl Elution Buffer / ddH_2O

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

General Protocol for DNA/PCR Clean-up

1. Transfer the DNA/PCR solution to clear microcentrifuge tube.

2. For DNA/PCR solution volume exceeds 100 µl, add **500 µl Binding Buffer** per 100 µl PCR reaction mix.

   Tech. Note:

   a. For DNA/PCR solution < 100 µl, adjust the volume upto 100 µl by TE buffer (*p*H 7.5).

   b. The maximum volume of the column is 750 µl, if sample volume more than 750 µl, just loading the remaining solution again after first spin in step 3, repeat step 3 & 4.

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

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

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

5. Discard the filtrate, and then centrifuge for **3 min** at top speed to remove residual trace of ethanol, additionally incubate the 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 automated fluorescent DNA sequencing and oven incubation will increase DNA yield.

6. Place the column into a clean microcentrifuge tube. To elute DNA, add **30~50 µl Elution Buffer,** H_2O (*p*H > 7.0) or TE buffer (*p*H 8.0) into the column, incubate at room temperature for **1 min** to increase the yield of eluted DNA, and then spin for **1 min** at maximum speed (12,000 ~14,000 x *g*).

Tech. Note:

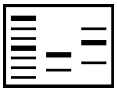
a. For larger DNA fragments (5~10 kb), use preheated (60~70^{0}C) **Elution Buffer** or H_2O or TE to elute.

b. When using water to elute, make sure the *p*H value is within 7.0~8.5. Lower *p*H may cause lower DNA recovery.

c. For automated fluorescent DNA sequencing, use only H_2O to elute DNA.

d. To check 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.

Flow Chart of Gel Extraction



Excise DNA fragment

100 mg + 100 µl Binding Buffer

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

Loading DNA mixtures (~ 700 µl) on Gene-Spin™ Column

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

+ 500 µl Binding Buffer

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

+ 700 µl Washing Buffer

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

Discard the filtrate

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

Dry the Gene-Spin™ Column

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

+ 30~50 µl 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) by a clean scalpel, cut the band into several small gel slices and weight the gel in a clean microcentrifuge tube.

2. Add **100 µl Binding Buffer** per **100 mg** agarose gel. Incubate sample at 60^{0}C for 5-15 min, and then vortex the sample briefly every 2~3 min until the gel slices are dissolved completely.

   Tech. Note:

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

   b. Adding more Binding Buffer could speed-up the melting time, but it will not increase the DNA recovery rate.

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

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

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

DNA for automated fluorescent DNA sequencing.

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

   Tech. Note: For automated fluorescent DNA 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 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 automated fluorescent DNA sequencing and oven incubation will increase DNA yield.

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

   Tech. Note:

   a. For larger DNA fragments (5~10 kb), use preheated (60~70^{0}C) **Elution Buffer** or H_2O or TE to elute.

   b. When using water to elute, make sure the *p*H value is within 7.0~8.5. Lower *p*H may cause lower DNA recovery.

c. For automated fluorescent DNA sequencing, use only H_2O to elute DNA.

d. To check 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 dissolving of agarose gel slices

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

⇨ *Time and temperature* - Check incubation temperature. Please cut gel into smaller slices before add **Binding Buffer**. Incubation (step 2) can be prolonged up to 20 min if the size of gel slices is not small enough. Vortex every 2 min and check integrity of the gel slice.

Q: No DNA yield

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

⇨ *Insufficient drying of the* ***Gene-Spin™ Column*** *with silica membrane* - Centrifuge 3 min at 12,000~14,000 x *g* before elution step to remove residual ethanol completely. And then, incubate the 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 larger DNA fragments (5~10 kb), use preheated 60~70^{0}C **Elution Buffer**, H_2O, or TE buffer to elute. And incubate on the silica membrane at room temperature for 3 min before centrifuge.

Q: Poor results with automated fluorescent DNA sequencing

⇨ *Use TE buffer for DNA elution* - use ethanol precipitation or this kit to purify DNA fragments again, 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 µl of the eluted DNA can be used per automated fluorescent DNA sequencing reaction.

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

Q: Poor restriction digestion

⇨ *Ethanol or salt carryover into the eluted DNA* - Use ethanol precipitation or keep the volume of DNA below 10% of the final reaction volume.

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

⇨ *Guanidine isothiocyanate contamination* - Low ratios do not necessarily indicate that the DNA will function poorly in downstream applications. Use ethanol precipitation 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* - Use ethanol precipitation, wash the pellet with 70% ethanol to reduce salt contamination.

Manufactured and distributed by
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