PCR Clean-Up & Gel Extraction Kit

Cat. No.: SN006-0300 Size: 300 Reactions
Cat. No.: SN006-0100 Size: 100 Reactions
Cat. No.: SN006-0004 Size: 4 Reactions
Sample: Up to 100 µl of the PCR Product
Up to 300 mg of DNA fragment from the agarose gel
Format: column form
Operation time: 15-20 minutes
Elution volume: 50-200 µl
Recovery: Up to 95%

Description

The PCR Clean-Up & Gel Extraction Kit provides a fast, easy, and cost-effective f system to isolate the DNA fragments from PCR reactions, agarose gels, or enzymatic reactions. The DNA fragments (100 base pairs-10 kilo base pairs) in the special buffers are bound by the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and the pure DNA is eluted with the Tris buffer or water without phenol extraction or alcohol precipitation. The DNA purified with the kits is suitable for any subsequent application, such as ligation and transformation, sequencing, restriction enzyme digestion, labeling, PCR, in vitro transcription, or microinjection. The entire procedure can be completed within 15-20 minutes.

Features

- Ready-to-use DNA for high performance in any downstream.
- Time flexibility.

Applications

- Quantity of DNA needed.
- Purity of DNA required.

Kit Contents

Contents	SN006-0300	SN006-0100	SN006-0004
Buffer B	80 ml X 2 bottles	60 ml X 1 bottle	2ml X 1 vial
Buffer W1	125 ml X 1 bottle	45 ml X 1 bottle	2 ml X 1 vial
Buffer W2 (Add ethanol)	25 ml X 2 bottles (100 ml X 2 bottles)	15 ml X 1 bottle (60 ml X 1 bottle)	300 µl X 2 vials (1.2 ml X 2 vials)
Buffer BE	30 ml X 1 bottle	10 ml X 1 bottle	1 ml X 1 vial
PG Column	50 pieces X 6 bags	50 pieces X 2 bags	4 pieces X 1 bag
Collection Tube	50 pieces X 6 bags	50 pieces X 2 bags	

Quality Control

The quality of the PCR Clean-Up & Gel Extraction Kit is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Ethanol (96-100%)
- 1.5 ml microcentrifuge tubes
- Water bath / Dry bath for Gel Extraction protocol needed

PCR Clean-Up & Gel Extraction Protocol

Step 1 Sample Preparation

PCR Clean Up

1. Add 500 µl of the Buffer B to the PCR product (up to 100 µl) and mix by vortex.

Gel Extraction

1. Excise the DNA fragment from the agarose gel.
2. Transfer the gel slice (up to 300 mg) to a 1.5 ml microcentrifuge tube.
3. Add 500 µl of the Buffer B to the sample and mix by vortex. Incubate at 60°C for 10 minutes (or until the gel slice has completely dissolved).
4. During the incubation, mix by vortexing the tube every 2-3 minutes. Cool the dissolved sample mixture to the room temperature.

Step 2 Binding

1. Place a PG Column in a Collection Tube. Apply the supernatant (from step 1) to the PG Column by decanting or pipetting.
2. Centrifuge at 14,000 x g for 30 seconds.
3. Discard the flow-through and place the PG Column back into collection tube.

* The maximum capacity of the PG Column reservoir is 800 µl. If the sample mixture is more than 800 µl, repeat the DNA Binding Step.

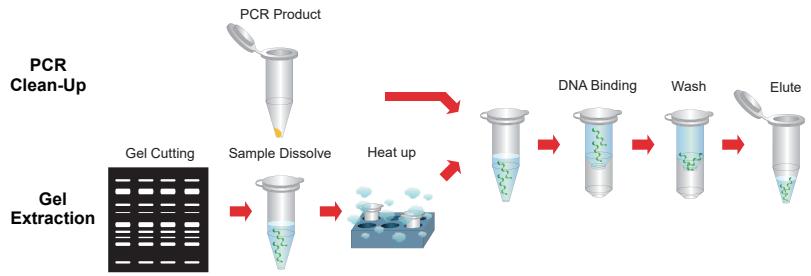
Step 3 Wash

Add 400 µl of the Buffer W1 into the PG Column.
Centrifuge at 14,000 x g for 30 seconds.
Discard the flow-through and place the PG Column back into the same collection tube.
Add 600 µl of the Buffer W2 (ethanol added) into the PG Column.
Centrifuge at 14,000 x g for 30 seconds.
Discard the flow-through and place the PG Column back into the same collection tube.
Centrifuge at 14,000 x g again for 2 minutes to remove the residual Buffer W2.

Step 4 Elution

To elute the DNA, place the PG Column in a clean 1.5 ml microcentrifuge tube.
Add 50-200 µl of the Buffer BE or H_2O (pH is between 7.0 and 8.5) to the center of each PG Column, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

NOTE: Check the buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did DNA fragments isolation with the kit.

Problem	Cause	Solution
Low yields of DNA	Buffer B with the incorrect ratio added to the amplification reaction	Verify that an equal volume of the Buffer B was added to the reaction.
	96-100% ethanol not used	Add ethanol (96-100%) to the Buffer W2 before use.
	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use the new glass and plastic wares, and wear gloves
	Column overloaded	Decrease the loading volume or lower the culture density.
	Dissolved incompletely	Increase time for the Gel Extraction Step until the gel slice has completely dissolved. Use an equal volume of the Buffer B and/ or low-melting-point agarose gels.
	Incorrect elution conditions	Ensure that the Buffer BE or ddH_2O is added into the center of the PG Column.
	Recovery buffer volume too small	Increase the amount of the Buffer BE to at least 50 µl for use.
Inhibition of downstream enzymatic reactions	TE buffer used for DNA elution	Use the ethanol to precipitate the DNA, or repurify the DNA fragments and elute with the nuclease-free water.
	Presence of residual ethanol in plasmid	Remove the ethanol in the hood briefly. Following the Wash step, dry the PG Column with additional centrifugation at 14-16,000 x g for 2 minutes.
DNA passed through in the flow-through or wash fraction	Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to the instructions.
Purified DNA floats out of wells while running in agarose gel	Traces of ethanol not completely removed from the column	Make sure that no residual ethanol remains in the membrane before eluting the plasmid DNA. Re-centrifuge if necessary.

Related Ordering Information

Cat. No.	Description	Size
SM101-0500	*Taq* DNA polymerase	500 U
SM200-0100	PCR SUPERMIX	100 Reactions
SM201-0100	Hot Start SUPERMIX	100 Reactions
SM255-0100	Ultrapure Proteinase K	100 mg
SA001-0500	AGAROSE Tablet, 0.5g	100 Tablets
SL001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml
SD003-R600	100 bp DNA Ladder H3 RTU	600 µl
SD010-R600	1 Kb DNA Ladder RTU	600 µl
SD013-R600	XLarge DNA Ladder RTU	600 µl
ST040-4000	100 mM dNTP Set	4 x1 ml
ST046-1000	100 mM dNTP Set	4 x 250 µl
ST025-1000	2.5 mM dNTP Mix	1 ml
ST010-1000	10 mM dNTP Mix	1 ml

Caution

- Buffers B and W1 contain irritants. Wear gloves when handling these buffers.
- When using 300 reaction assays, add 100 ml of the ethanol (96-100%) to each bottle of the Buffer W2, and shake before use.
- When using 100 reaction assays, add 60 ml of the ethanol (96-100%) to the Buffer W2, and shake before use.
- When using 4 reaction assays, add 1.2 ml of the ethanol (96-100%) to each vial of the Buffer W2, and shake before use.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.