PCR Clean-Up & Gel Extraction Kit

Cat No. PDC01-0100(S)
Size: 100 Reactions/4 Reactions

Description

The **PCR Clean-Up & Gel Extraction Kit** provides a cost-effective system for the fast and easy isolation of the DNA fragments from PCR reactions, agarose gels, or enzymatic reactions. The DNA fragments (100bp~10Kb) 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.

Kit Contents

Contents	PDC01-0100	PDC01-0100S
Buffer DE	60 ml	2 ml
Buffer W1	45 ml	2 ml
Buffer W2 (Add ethanol)	15 ml (60 ml)	300 µl x2 (1.2 ml x2)
Buffer RE	10 ml	1 ml
Column PG	100 pcs	4 pcs
Collection Tubes	100 pcs	4 pcs

Required Sample

Cell	Required Sample
PCR Product	100 µl
Agarose gel slice	300 mg

Required Materials

- Ethanol (96~100%)
- 1.5 ml microcentrifuge tubes
- Water bath / Dry bath

Buffer Preparation

- Add 60 ml of the ethanol (96~100%) to the Buffer W2 and shake before use.

Protocol

PCR Clean-Up

Step 1 Sample Preparation

1. Add 500 µl of the Buffer DE to 100 µl of the PCR product and mix by vortex.

Step 2 Binding

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

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

Step 3 Wash

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

Step 4 Elution

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

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

Gel Extraction

Step 1 Sample Preparation

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

Step 2 Binding

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

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

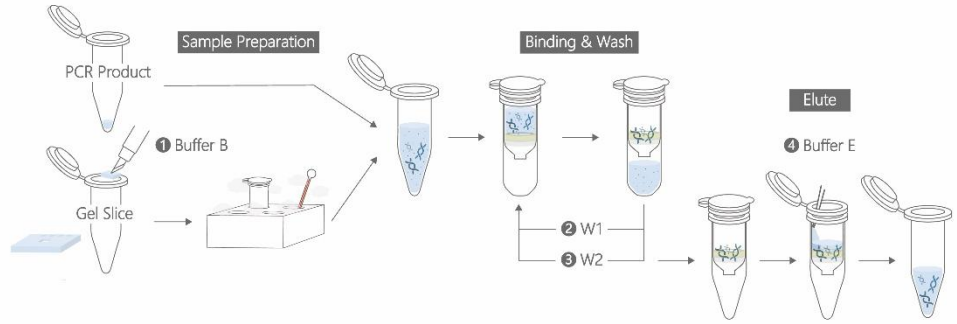
Step 3 Wash

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

Step 4 Elution

1. To elute the DNA, place the Column PG in a clean 1.5 ml microcentrifuge tube.
2. Add 50-100 µl of the Buffer RE or H_2O (pH is between 7.0 and 8.5) to the center of each Column PG, let it stand for 2 minutes, and centrifuge at 14,000 x g for 2 minutes.

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



Troubleshooting

Problem	Cause	Solution
Low yields of DNA	Buffer DEwith the incorrect ratio added to the amplification reaction	Verify that an equal volume of the Buffer DE 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. If overloaded, separate into 2 columns. If the DNA fragments are more than 300 mg, separate the gel slice into two microcentrifuge tubes.
	Dissolved incompletely	Increase time for the Gel Extraction Step until the gel slice has completely dissolved. Use an equal volume of the Buffer DE and or low-melting-point agarose gels.
	Incorrect elution conditions	Ensure that the Buffer RE or dd$H_2O$is added into the center of the Column PG.
	Recovery buffer volume too small	Increase the amount of the Buffer RE 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 EtOH in the hood briefly. Following the Wash step, dry the Column PG with additional centrifugation at 14~16,000 x g for 2 minutes.
DNA passed through in the flow-through or wash fraction	Column overloaded	Check the loading volume. If overloaded, separate into two columns.
	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.

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Check Buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
- Buffers B and W1 contain irritants. Wear gloves when handling these buffers.
- Add 60 ml of the ethanol (96~100%) to the Buffer W2 and shake before use.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.