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INSTRUCTION MANUAL

96 Well PCR Cleanup Kit

IB47035 (2 x 96 well plates/kit)

IB47040 (4 x 96 well plates/kit)

IB47050 (10 x 96 well plates/kit)

Advantages

Sample: up to 100 µl of PCR products per well

Fragment Size: 70 bp-20 kb

Recovery: up to 95%

Format: PCR Cleanup 96 Well Binding Plates for efficient vacuum filtration and centrifugation

Operation Time: 20 minutes Elution

Volume: 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume

Kit Storage: dry at room temperature (15-25ºC)

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Introduction

96 Well PCR Cleanup Kits were designed to recover or concentrate DNA fragments from PCR or other enzymatic reactions using an efficient 96 well binding plate system. Chaotropic salt is used to denature enzymes. DNA fragments in the chaotropic salt are bound by the glass fiber matrix in each well of the binding plate. Contaminants are removed with a Wash Buffer (containing ethanol) and the purified DNA fragments are eluted by a low salt Elution Buffer, TE or water. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation and the purified DNA is ready for use in subsequent reactions.

Quality Control

The quality of the 96 Well PCR Cleanup Kit is tested on a lot-to-lot basis by purifying DNA fragments of various sizes from PCR products. The purified DNA is analyzed by electrophoresis.

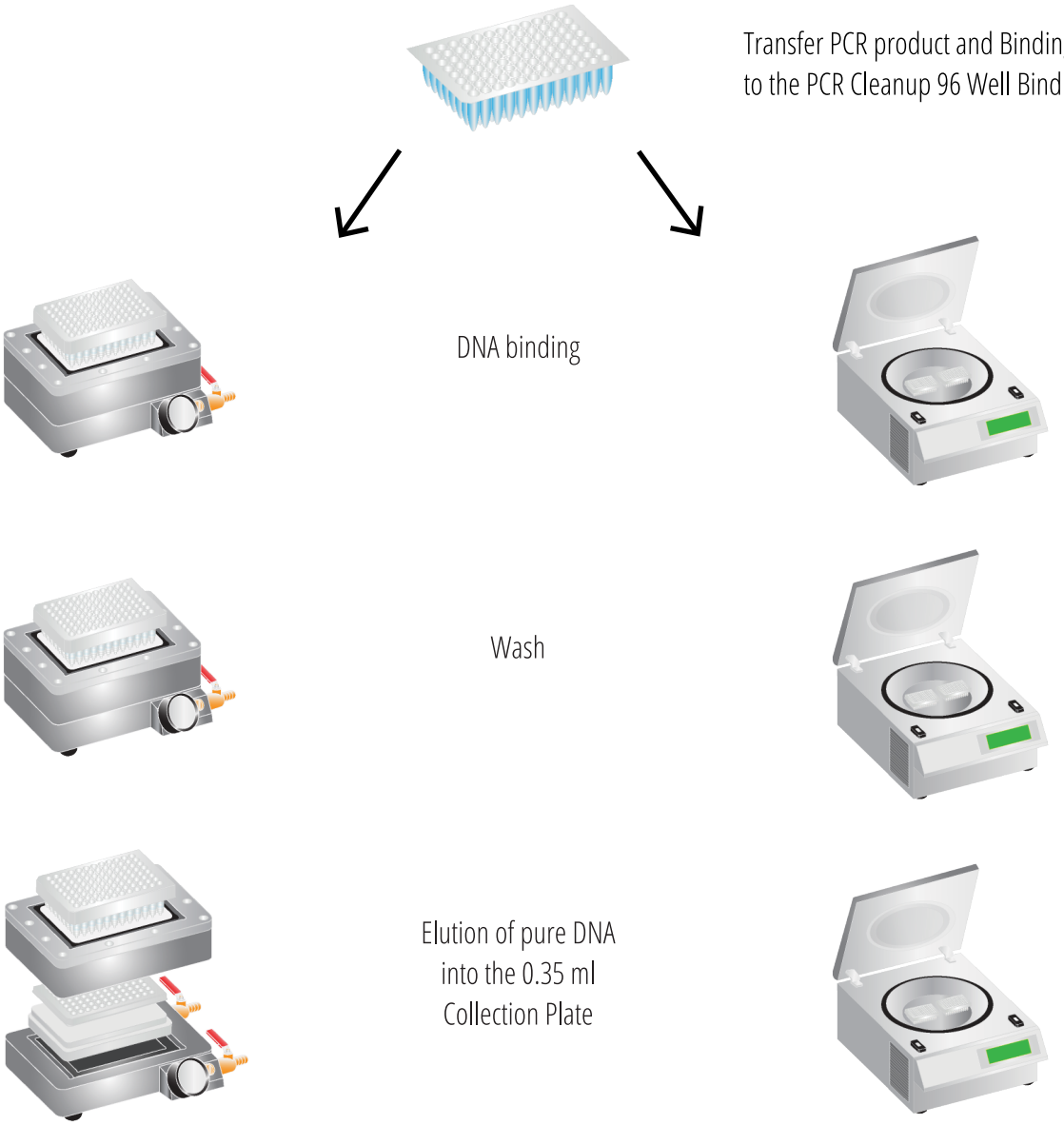
Kit Components

Component	IB47035	IB47040	IB47050
Binding Buffer	40 ml	80 ml	240 ml x 1
Wash Buffer1 (Add Ethanol)	50 ml (200 ml)	50 ml x 2 (200 ml x 2)	50 ml x 5 (200 ml x 5)
Elution Buffer	30 ml	60 ml	100 ml
PCR Cleanup 96 Well Binding Plates	2	4	10
0.35 ml Collection Plates	2	4	10
Adhesive Film	4	8	10

[1]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.

Binding Buffer contains guanidine thiocyanate. During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

Quick Vacuum and Centrifuge Protocol Diagram



96 Well PCR Cleanup Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. 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.
2. It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup. Additional Requirements absolute ethanol, 96 Deep Well Plate (optional), Square-well Block (optional)

PCR Cleanup Vacuum Protocol Procedure

1. Vacuum Manifold Preparation

Place the waste tray on the manifold base then place the binding top plate on the manifold base. Place the **PCR Cleanup 96 Well Binding Plate** in the binding top plate aperture. Seal unused wells of the **PCR Cleanup 96 Well Binding Plate** with **Adhesive Film** then attach the vacuum manifold to a vacuum source.

2. DNA Binding

Add **3 volumes of Binding Buffer to 1 volume of PCR sample** then mix by pipetting. Transfer the sample mixture to each well of the **PCR Cleanup 96 Well Binding Plate** (E.g. Add 150 µl of Binding Buffer to 50 µl of PCR sample). Apply vacuum at 15 inches Hg until samples pass through completely (approximately 10 seconds) then turn off the vacuum.

3. Wash

Add **500 µl of Wash Buffer (make sure ethanol was added)** to each well of the **PCR Cleanup 96 Well Binding Plate**. Let stand for 1 minute. Apply vacuum at 15 inches Hg until Wash Buffer passes through completely (approximately 10 seconds) then turn off the vacuum. **Add 500 µl of Wash Buffer (make sure ethanol was added)** to each well. Apply vacuum at 15 inches Hg until Wash Buffer passes through completely. Continue to apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

4. Elution

Remove the **PCR Cleanup 96 Well Binding Plate** from the binding top plate aperture and blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place the collection plate spacer on the manifold base. Place a **0.35 ml collection plate** on top of the collection plate spacer. Place the binding top plate back on the manifold base then place the **PCR Cleanup 96 Well Binding Plate** back in the binding top plate aperture. Add **60-80 µl of Elution Buffer**[1], TE[2] or water[3] into the **CENTER** of each well of the **PCR Cleanup 96 Well Binding Plate**. Let stand for at least 3 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes. Seal the **0.35 ml Collection Plate** with **Adhesive Film** then store the purified DNA at -20°C.

NOTE! The average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the well matrix and is completely absorbed.
[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the well matrix and is completely absorbed.
[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

PCR Cleanup Centrifuge Protocol Procedure

1. DNA Binding

Place the **PCR Cleanup 96 Well Binding Plate** on a 96 Deep Well Plate or a standard Square-Well Block. Add **3 volumes of Binding Buffer to 1 volume of the PCR sample** then mix by pipetting. Transfer the sample mixture to each well of the **PCR Cleanup 96 Well Binding Plate**. (E.g. add 150 µl of Binding Buffer to 50 µl PCR sample). Centrifuge the **PCR Cleanup 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **PCR Cleanup 96 Well Binding Plate** back on the 96 Deep Well Plate.

2. Wash

Add **500 µl of Wash Buffer (make sure ethanol was added)** to each well of the **PCR Cleanup 96 Well Binding Plate**. Let stand for 1 minute. Centrifuge the **PCR Cleanup 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **PCR Cleanup 96 Well Binding Plate** back on the 96 Deep Well Plate. Add **500 µl of Wash Buffer (make sure ethanol was added)** to each well of the **PCR Cleanup 96 Well Binding Plate**. Centrifuge the **PCR Cleanup 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the **PCR Cleanup 96 Well Binding Plate** back on the 96 Deep Well Plate. Centrifuge the **PCR Cleanup 96 Well Binding Plate** and 96 Deep Well Plate together at 3,000 x g for an additional 10 minutes to dry the membrane.

3. Elution

Remove the **PCR Cleanup 96 Well Binding Plate** from the 96 Deep Well Plate then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the **PCR Cleanup 96 Well Binding Plate** on a **0.35 ml Collection Plate**. Add **60-80 µl of Elution Buffer**[1], TE[2] or water[3] to the center of each well of the **PCR Cleanup 96 Well Binding Plate**. Let stand for at least 3 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the **PCR Cleanup 96 Well Binding Plate** and **0.35 ml Collection Plate** together at 3,000 x g for 5 minutes to elute the purified DNA. Seal the **0.35 ml Collection Plate** with **Adhesive Film** then store the purified DNA at -20°C

NOTE! The average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the well matrix and is completely absorbed.
[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the well matrix and is completely absorbed.
[3]If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Troubleshooting

Low Yield

Incomplete Wash Buffer preparation.

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.

Ensure that Elution Buffer, TE or water is added into the **CENTER** of the well matrix and is completely absorbed. If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

96 Well PCR Cleanup Kit Functional Test Data

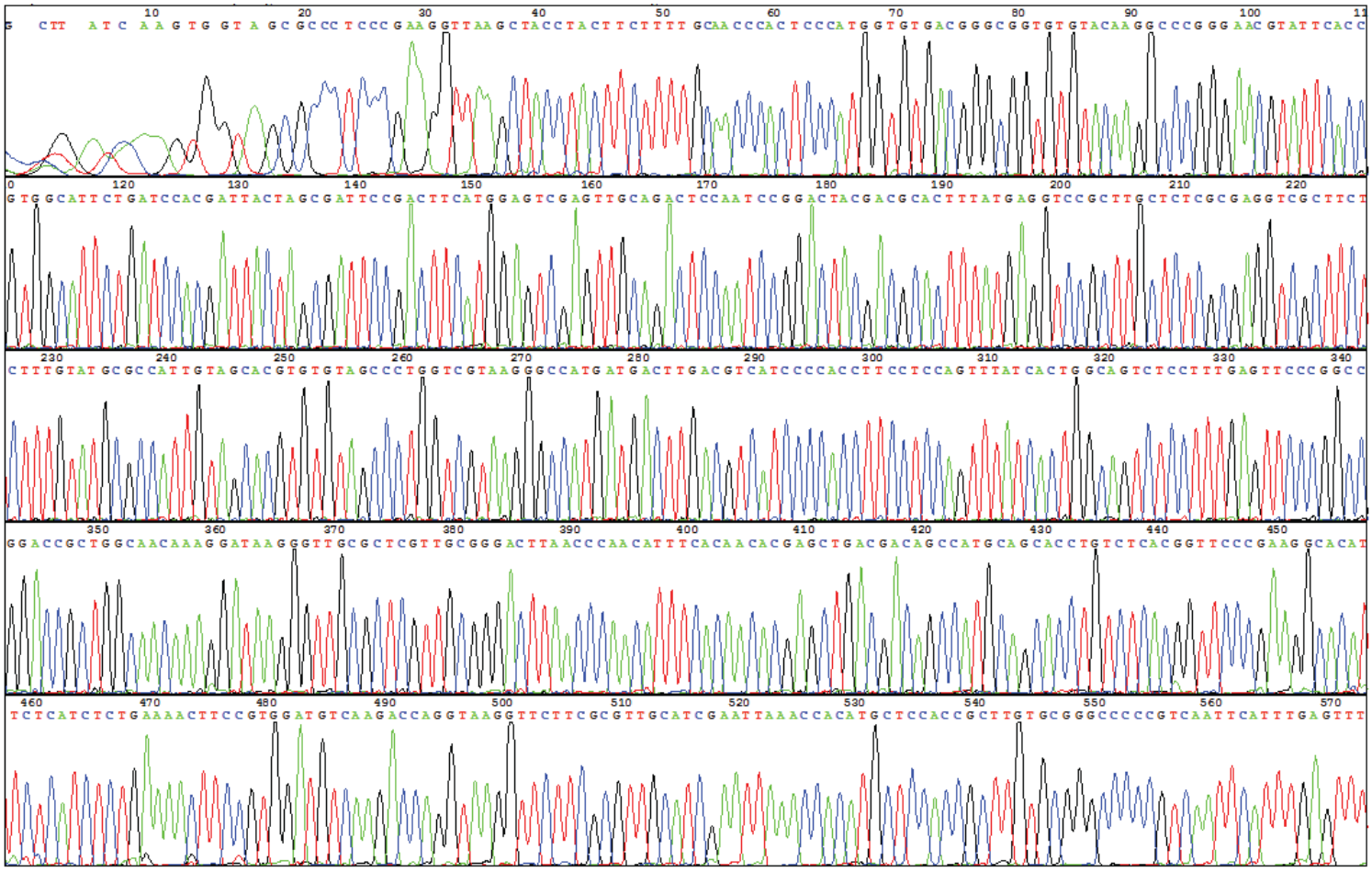


Figure 1. Sequencing data of PCR product (E. coli 16S ribosomal DNA fragment) purified using the 96 Well PCR Cleanup Kit.

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