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Product Information

GenElute™ 96 Well PCR Clean-Up Kit

Catalog Numbers **PCR9601, PCR9604**

TECHNICAL BULLETIN

Product Description

The GenElute 96 Well PCR Clean-Up Kit offers a simple, rapid and cost-effective solution for high-throughput purification of PCR products. The kit provides the necessary reagents and consumables for purification of up to 15 µg of highly pure PCR product. DNA recovery of 75 – 90% is obtained from DNA fragments of 100 to 10,000 bp while removing primers, primer-dimers, nucleotides, salts and polymerase. Following the procedure, 96 samples can be processed in 45 minutes and less than 96 samples by using a rubber pad or self adhering foil to cover wells that are not used.

Process Overview

After the PCR reaction is complete, the reaction volume is adjusted before the addition of the Binding Solution. The samples are then transferred to the Binding Plate where the PCR reaction is captured by the silica membrane. The bound PCR product undergoes subsequent wash steps using Wash Solution to remove primers, primer-dimers, salts, nucleotides and proteins (e.g. polymerases, BSA). Finally, the purified PCR product is eluted in Elution Solution or water and ready for immediate use in downstream applications.

Storage

Store the kit at room temperature. If any reagent forms a precipitate, see Preparation Instructions that follow.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Binding Solution contains chaotropic salt. Wear gloves and safety glasses when handling this solution or any reagent provided with the kit.

Reagents Provided	Catalog Number	PCR9601 96 Preps	PCR9604 4x96 Preps
Binding Solution	B8813	30 mL	2x75 mL
Wash Solution, Concentrate	W3145	100 mL	200 mL
Elution Solution	E5536	25 mL	125 mL
Binding Plate	B8938	1 each	4 each
Wash Plate	W3270	1 each	4 each
Elution Plate, U-Bottom	E5911	1 each	4 each

Reagents and Equipment Required but Not Provided

- GenElute 96 Vacuum Manifold, Catalog No. VM01
- Vacuum Source (vacuum of 200-400mbar)
- GenElute Vacuum Regulator, Catalog No. VM02 (recommended)
- 95-100% Ethanol, Catalog No. E7148, E7023 or 459836
- Nuclease free water, Catalog No. W4502
- Tris HCl buffer, pH 7.0, Catalog Number T1819
- Centrifuge capable of >4,000*g* (optional)

Preparation Instructions
Before beginning the procedure, complete the following:

Dilute the Wash Solution, Concentrate with 95-100% ethanol prior to use: add 400 mL for the 1 x 96 prep kit and 800 mL for the 4 x 96 prep kit.

Vacuum Manifold Use

Establish a reliable vacuum source for the GenElute Vacuum Manifold. The manifold may be used with a vacuum pump, house vacuum, or water aspirator. A vacuum of 200-400 mbar (pressure difference) is recommended. The use of a GenElute Vacuum Regulator (VM02) is also recommended. Alternatively, adjust vacuum during the purification such that the sample flows through the column with a rate of 1-2 drops per second.

Procedure

1. **Perform PCR reactions.**

2. **Adjust the volume of reaction mix.** For PCR reaction volumes below 100 µL, add Tris HCl buffer (10 mM, pH 7.0), nuclease-free water (pH 7.0-7.5), or Elution Solution to adjust the reaction mixture to a final volume of 100 µL.

3. **Prepare the GenElute Vacuum Manifold.**

   a. The spacers are inserted notch side up into the grooves located on the short sides of the manifold.
   b. The waste container is placed into the manifold base.
   c. Insert Binding Plate into the manifold lid.
   d. Close the vacuum manifold's valve, check and adjust the vacuum pressure (pressure difference -200 mbar)

4. **Dispense Binding Buffer to the Binding Plate**. Add 2 volumes of Binding Solution for 1 volume of the PCR reaction and mix. For example, add 200 µL of Binding Solution to a 100 µL PCR reaction.

5. **Transfer PCR samples onto the Binding Plate and mix.** Mix by pipetting up and down 5 times.

6. **Bind DNA**. Apply vacuum and press down the plate slightly until flow-through starts. Transfer the solution into the Binding Plate and allow the lysate to clear. Once the lysate is completely filtered release the vacuum by closing the valve.

7. **Apply Wash Solution.** Add 900 µL Wash Solution (with ethanol added) to each well of the Binding Plate. Apply vacuum and press down the Binding Plate slightly until flow-through starts. Allow the buffer to pass completely through the columns.

8. **Repeat Wash Solution**. Add 900 µL Wash Solution (with ethanol added) to each well of the Binding Plate. Apply vacuum and press down the Binding Plate slightly until flow-through starts. Allow the buffer to pass completely through the columns.

9. **Remove Wash Plate.** After the final wash, close the valve, release the vacuum and remove the Binding Plate. Remove manifold lid, Wash Plate, and waste container from the GenElute Vacuum Manifold.

10. **Dry Binding Plate.** Remove any residual Wash Solution from the Binding Plate. If necessary, tap the outlets of the Binding Plate on clean paper sheets (supplied with the Wash Plate) or soft tissue until no drops come out. Insert the Binding Plate into the lid and close the manifold. Apply vacuum of 300-400 mbar (pressure difference) for at least 10 min to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

    Note: The ethanol in Wash Solution inhibits enzymatic reactions and has to be removed completely before DNA elution.

11. **Insert Elution Plate, u-bottom.** Insert the Elution Plate onto the spacers inside the manifold base. If eluting into microtiter plates, spacers should be inserted into the manifold base before inserting the microtiter plate.

12. **Elute highly pure PCR-product DNA.** Add 75-150 µL of Elution Solution (5 mM Tris-HCl, pH 8.5) to the center of each well of the Binding Plate. Incubate for 1 to 3 minutes at room temperature, apply vacuum, and collect the eluted DNA. After the elution buffer has passed through the columns, close the valve and release the vacuum. Remove the elution plate containing eluted DNA. Prior to storage, plates should be sealed with adhesive foil.

Elution Spin Method

Elution of purified DNA in a centrifuge may be necessary when higher concentrations of DNA are required for downstream applications. Using a centrifuge allows reduction of the dispensed volume to 50-75 µL giving a DNA concentration of 70-200 ng/µl depending on Elution Buffer volume and fragment length.

1. After the final washing step with Wash Solution, remove the Binding Plate from the GenElute Vacuum Manifold and tap on a paper sheet to remove residual Wash Solution.

2. Place the Binding Plate on top of a Square-well Block or similar (not provided in the kit) and load the plate sandwich to a suitable centrifuge. Centrifuge for 10 min at maximum speed (> 4,000 x *g*, optimal 5,800 x *g*) to dry the membrane and the outlets of the binding plate.

Note: We recommend using a centrifuge, e.g. Qiagen/Sigma 4-15, Jouan KR4i, Kendro-Heraeus Multifuge 3/3-R, HighplateTM rotor, Beckman Coulter, Allegra R, with a swing-out rotor which is capable of accommodating Binding Plate/Square-well Block sandwich (bucket height: 85 mm).
Do not use a microtiter plate as a support for Binding Plate. Microtiter plates like the provided Elution Plate, u-bottom may break when centrifuging at >2,500 x g

3. Insert the Binding Plate onto a new Square-well Block. Remove the self-adhering PE foil and dispense 50 -75 µL Elution Solution directly in the center of each well of the Binding Plate and incubate for 1 to 3 minutes at room temperature.

Note: Elution in 96-well PCR plates is possible. In order to stabilize the PCR plate during centrifugation, use a suitable plate adapter. Alternatively, insert the PCR plate in a Square-well Block. Place Binding Plate on top and centrifuge the whole assembly for elution.

4. Centrifuge for 2 minutes at maximum speed (> 4,000 x *g*, optimal 5,800 x *g*) to collect the DNA. Remove the Square-well Block or Round-well Block containing eluted DNA and seal with adhesive PE foil until use.

Reference

1. Vogelstein, B., and Gillespie, D. *Proc. Natl. Acad. Sci. USA* 76, 615-619.

Troubleshooting Guide

Problem	Cause	Solution
Poor DNA yield	No ethanol added to Wash Solution, Concentrate, ethanol evaporated	Yields will vary greatly among different cell and tissue types. See “Expected Yield” in the Results section.
	Elution conditions are not optimal	If possible, use a slightly alkaline elution solution like the one included in this kit (5 mM Tris-HCl, pH 8.5). When using nuclease-free water for elution, make sure the pH value is 8.5. Elution efficiencies drop dramatically with buffers < pH 7.
	Elution Solution volume is insufficient	Optimal elution is achieved for an elution buffer volume of 100-150 µL. Do not use less than 75 µL elution buffer.
Suboptimal performance of PCR product in sequencing reactions, problems with downstream applications	Carry over of ethanol	Be sure to remove all of ethanolic wash solution after the final washing step. Dry the Binding Plate for at least 10 min with maximum vacuum.
	Elution of PCR products with buffer containing EDTA	EDTA may inhibit enzymatic reactions like DNA sequencing. Repurify the PCR products and elute with Elution Solution included in this kit or nuclease-free water. Alternatively, the DNA may be precipitated with ethanol and redissolved in Elution Solution or nuclease-free water.
	Not enough DNA used in sequencing reactions	Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.
	Contamination of PCR product preparation with ethanol	Insufficient drying after final washing step with wash solution. Remaining ethanol may cause problems with downstream applications like DNA sequencing or loading of samples onto agarose gel.
	Eluted DNA contains residual primers/primer dimers	Minimized amount of primers in PCR reaction mixture. Make sure that the ratio of binding solution: PCR reaction is 2:1.

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