

Overview and Intended Use

The PowerPrep™ Express 96 PCR Purification Kit is designed for the rapid purification of PCR products. Binding solution is added to the amplification reaction and the mixture is applied to a 96-well DNA Binding Plate where the double-stranded DNA is selectively adsorbed. DNA polymerases, buffer, unreacted primers and dNTPs are removed with an alcohol-containing wash buffer. Since the DNA is eluted at high concentration in TE Buffer, no precipitation is necessary. All solutions are passed through the cartridge with brief centrifugations or vacuum filtrations, allowing the completion of the protocol in <10 min. This product eliminates the use of hazardous chemicals such as phenol and chloroform.

- Up to 5 µg of double-stranded DNA from 80 bp to 20 kb can be purified with the PowerPrep™ Express 96 PCR Purification Kit.
- DNA purified with this kit is of high quality and is suitable for automated fluorescent DNA sequencing, manual DNA sequencing, amplification reactions, restriction mapping, cloning and labeling.
- The PowerPrep™ Express 96 PCR Purification Kit is also suitable for the purification of double-stranded DNA from restriction endonuclease reactions, labeling reactions, and other DNA modifying reactions.

Important Information

The product you have received is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic or therapeutic application. Uses other than the labeled intended use may be a violation of applicable law.

Precautions

Warning: This product contains hazardous reagents. It is the end-user's responsibility to consult the applicable MSDS(s) before using this product. Disposal of waste organics, acids, bases and radioactive materials must comply with all appropriate federal, state and local regulations. If you have any questions concerning the hazards associated with this product, please call OriGene at (301) 340-3188.

Critical Parameters

- Use volumes precisely as indicated in the protocol.

Safety and Use Statement

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

Components included with this kit:

	NP100018	NP100019
COMPONENT NAME	4 x 96 RXN	12 x 96 RXN
Binding Solution (D1)	160 ml	480 ml
Wash Buffer (D2)	80 ml	2 x 120 ml
TE Buffer (TE)	30 ml	85 ml
DNA Binding Plate	4 each	12 each
Wash Plate (750 µl well capacity)	4 each	12 each
Collection Plate (350 µl well capacity)	4 each	12 each

Storage Conditions

Store all reagents at room temperature.

Additional Materials Required

- 95-100% ethanol
- centrifuge or vacuum manifold capable of handling 96 well plates
- 65 to 70°C water bath or heat block

Advance Preparations

Add 95-100% ethanol to Wash Buffer (D2) according to the instructions on the label of the bottle (D2), then store Wash Buffer (D2) at room temperature.

CENTRIFUGATION PROTOCOL

Before Beginning:

- Verify that ethanol has been added to Wash Buffer (D2) (See Advance Preparations.)
- Preheat TE Buffer to 65-70°C.

Perform all centrifugations at room temperature (20° – 25° C).

1. **Sample Preparation:** Add 400 µl of Binding Solution (D1) to the amplification reaction and mix thoroughly. Removal of oil is not necessary.
Note: Use 400 µl of Binding Solution (D1) for amplification reactions ≤100 µl. For amplification reactions ≥100 µl, adjust the volume of Binding Solution (D1) to a ratio of D1: amplification reaction of ≥ 4:1 (v/v).
 2. **Sample Loading:** Place the DNA Binding Plate on top of the Wash Plate supplied with the kit. Load the samples from step 1 into the DNA Binding Plate. Centrifuge the plates at 1,000 x g (2500 rpm using a Sorvall tabletop centrifuge RT6000B with an H1000B swinging bucket rotor) for 3 minutes. Discard the flow-through in the Wash Plate.
 3. **Plate Wash:** Replace the DNA Binding Plate on top of the Wash Plate and add 700 µl of Wash Buffer (D2) containing ethanol to each well of the plate. Centrifuge the plates at 1,000 x g (2500 rpm using a Sorvall tabletop centrifuge RT6000B with an H1000B swinging bucket rotor) for 5 minutes. Discard the flow-through, reposition the plates as before and spin the plates again at 1,000 x g for 2 minutes to remove any residual ethanol.
 4. **DNA elution:** Place a Collection Plate under the DNA Binding Plate. Add 50 µl of TE buffer* directly to the center of the wells of the DNA Binding Plate. Incubate at room temperature for 3 minutes, then centrifuge at 1,000 x g for 3 minutes.
***Note:** Room temperature TE buffer (TE) may be used for elution, but optimal recovery is obtained with TE warmed to 65° - 70°C, especially for larger PCR products.
 5. **Recovery and Storage:** Purified DNA will be in the wells of the Collection Plate. The DNA can be stored in the Collection Plate by sealing the plate with an aluminum plate sealer or the DNA can be transferred to microcentrifuge tubes. Store the DNA at –70°C until used.
1. **Sample Preparation:** Add 400 µl of Binding Solution (D1) to the amplification reaction and mix thoroughly. Removal of oil is not necessary.
Note: Use 400 µl of Binding Solution (D1) for amplification reactions ≤100 µl. For amplification reactions ≥ 100 µl, the volumetric ratio of D1 and amplification reaction should be 4:1 (v/v).
 2. **Sample Loading:** Place the DNA Binding Plate on top of the vacuum manifold. Load the samples from step 1 into the DNA Binding Plate (if the entire plate is not used, we recommend covering the unused wells with aluminum plate sealers, to maintain vacuum pressure. Apply vacuum to the plate for 2 minutes.
 3. **Plate Wash:** Add 700 µl of Wash Buffer (D2) containing ethanol to each well of the plate. Apply vacuum for 2 minutes. Discard the flow-through, reposition the plates as before and apply vacuum for an additional 2 minutes to remove any residual ethanol.
Note: For high throughput automated processing with a vacuum system, do not use the Wash Plate. Apply vacuum for two minutes and allow the waste to drain through the vacuum system. When the Wash Plate is not used, the wells should dry sufficiently with a single application of vacuum for 2 minutes.
 4. **DNA elution:** Place a Collection Plate (supplied with kit) into the vacuum manifold, replace the cover and position the DNA Binding Plate as before. It is very important that the Collection Plate be positioned so that the drip directors on the DNA Binding Plate are as close as possible to the Collection Plate.
Note: A Wash Plate (or something of similar size) can be placed under the Collection Plate to raise the height of the Collection Plate to ensure contact between the DNA Binding Plate and the Collection plate. If the Collection Plate is not in contact with the DNA Binding Plate, cross contamination may occur.
 5. Add 50 µl of TE buffer* directly to the center of the wells of the DNA Binding Plate. Incubate at room temperature for 3 minutes, then apply vacuum for 2 minutes.
***Note:** Room temperature TE buffer (TE) may be used for elution, but optimal recovery is obtained with TE warmed to 65° - 70°C, especially for larger products.
 6. **Recovery and Storage:** Purified DNA will be recovered in the wells of the Collection Plate. The DNA can be stored in the Collection Plate by sealing the plate with an aluminum plate sealer or the DNA can be transferred to microcentrifuge tubes.
Store the DNA at –20°C until used.

VACUUM PROTOCOL

Before Beginning:

- Verify that ethanol has been added to Wash Buffer (D2) (See Advance Preparations.)
- Preheat an aliquot of TE buffer to 65°-70°C
- The vacuum manifold used must be capable of achieving vacuum of at least –15 in. Hg.

Problem	Possible Cause	Suggested Solution
Low DNA Yield	Poor Amplification	Verify yield from amplification reaction by electrophoresis of an aliquot on an agarose gel.
	Incorrect DNA binding conditions	Use ratios of solution D1:amplification reaction of $\geq 4:1$ (v/v).
	Incomplete DNA elution	Ensure that TE Buffer (TE) is warmed to 65 to 70°C. This is especially critical for DNA > 5kb.
DNA concentration too low		Reduce the elution volume to 30 μ l. This will give higher DNA concentrations, but will also decrease DNA yield.
		Precipitate eluted DNA and dissolve in a smaller volume of TE Buffer.
Inhibition of enzymatic reaction	Ethanol in DNA eluate	Discard wash buffer flow-through prior to the second centrifugation in step 3.