ORIGENE
Your Gene Company

PowerPrep™ Express PCR Purification Kit

Store at RT

Catalog No. NP100015 (11458-015) 50 Reactions
Catalog No. NP100016 (11458-023) 250 Reactions

Overview and Intended Use

The PowerPrep™ Express PCR Purification Kits are designed for rapid purification of PCR products. Binding solution is added to the amplification reaction and the mixture is applied to a spin cartridge containing silica-based membranes where the double-stranded DNA is selectively adsorbed (1). Adsorption to the membranes is influenced by buffer composition and temperature. 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, allowing the completion of the protocol in <10 min. This product eliminates the use of hazardous chemicals such as phenol and chloroform.

- Up to 15 µg of double-stranded DNA from 80 bp to 20 kb can be purified.
- DNA purified is high quality and is suitable for automated fluorescent DNA sequencing, manual DNA sequencing, amplification reactions, restriction mapping, cloning, and labeling.
- The kit is also suitable for the purification of double-stranded DNA from restriction endonuclease reactions, labeling reactions, and other DNA modifying reactions.

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 (888)-267-4436.

Reference

1. Vogelstein, B. and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA 76,* 615.

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: Store all components at room temp.

	NP100015	**NP100016**
Component Name	50 rxn	250 rxn
Binding Solution	20 mL	100 mL
Wash Buffer	12 mL	55 mL
TE Buffer	15 mL	15 mL
Spin Cartridges, Wash Tubes and Recovery Tubes	50 each	250 each

Critical Parameters

Use volumes precisely as indicated in the protocol.

Additional Materials Required

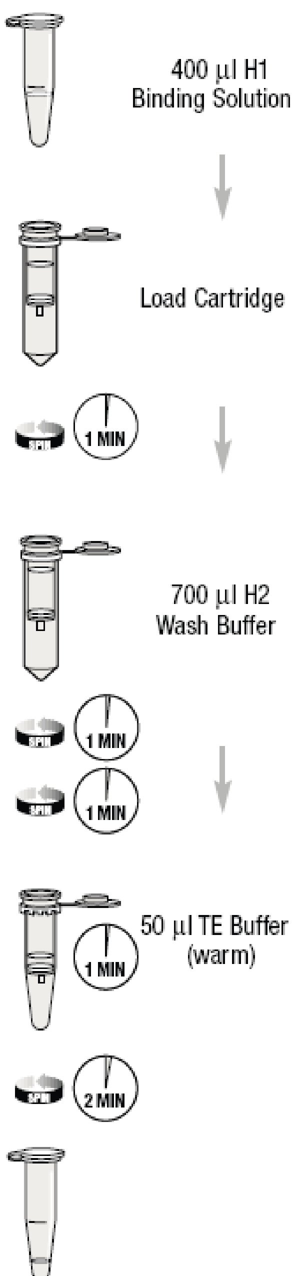
- 95-100% ethanol
- Microcentrifuge capable of reaching ≥ 12,000 x *g*
- 65 to 70°C water bath or heat block

Advance Preparations

Add 95-100% ethanol (EtOH) to the Wash Buffer according to the following instructions.

Volume of **Wash Buffer**	Volume of **EtOH** to add to Wash Buffer
12 mL	30 mL
55 mL	140 mL

These instructions are also on the label of the bottle. Mix well. Place a mark on the label to indicate that ethanol has been added



Centrifugation Protocol Insert

Perform all centrifugations at room temperature.

Before beginning: Preheat an aliquot of **TE Buffer** to 65 to 70°C. Verify that ethanol has been added to **Wash Buffer** (See Advance Preparations).

1. **Sample Preparation**: Add 400 µL of **Binding Solution** to the amplification reaction and mix thoroughly. Removal of oil is not necessary.

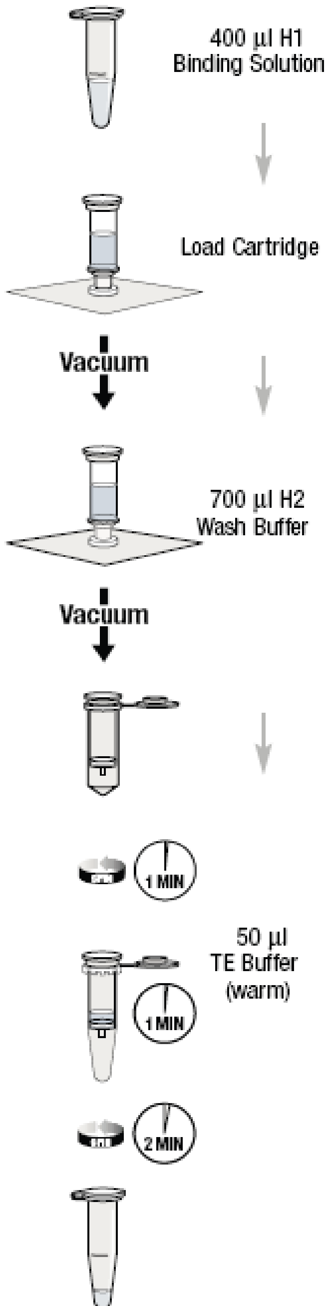
   NOTE: Use 400 µL of **Binding Solution** for amplification reactions ≤ 100 µL. For amplification reactions > 100 µL, adjust the volume of **Binding Solution** to a ratio of H1:amplification reaction of **≥**4:1 (v/v).

2. **Cartridge Loading**: Place a cartridge into a 2 mL wash tube. Load the sample from step 1 into the cartridge. Centrifuge the mixture in a microcentrifuge at ≥12,000 x *g* for 1 min. Discard the flow-through.

3. **Cartridge Wash**: Place the cartridge back into the 2 mL wash tube. Add 700 µL of **Wash Buffer** (containing ethanol) to the cartridge. Centrifuge at ≥12,000 x *g* for 1 min. Discard the flow-through. Centrifuge again at ~12,000 x *g* for 1 min to remove all residual wash buffer.

4. **DNA Elution**: Place the cartridge into a 1.5 mL recovery tube (supplied). Add 50 µL of warm **TE Buffer** directly to the center of the cartridge. Incubate at room temperature for 1 min, then centrifuge at ≥12,000 x *g* for 2 min.

NOTE: Room temperature TE Buffer may be used for elution, but optimal recovery is obtained with TE warmed to 65 to 70°C.



Vacuum Manifold Protocol Insert

Perform all centrifugations at room temperature.

Before beginning: Preheat an aliquot of **TE Buffer** to 65 to 70°C. Verify that ethanol has been added to **Wash Buffer** (See Advance Preparations).

1. **Sample Preparation**: Add 400 μL of **Binding Solution** to the amplification reaction and mix thoroughly. Removal of oil is not necessary.

NOTE: Use 400 μL of **Binding Solution** for amplification reactions > 100 μL. For amplification reaction > 100 μL, adjust the volume of **Binding Solution** to a ratio of H1: amplification reaction of ≥4:1 (v/v).

2. **Vacuum Manifold Preparation**: Attach the vacuum manifold to a vacuum source. Attach a cartridge to a luer extension on the vacuum manifold.

3. **Cartridge Loading**: Load the sample from step 1 into the cartridge. Apply vacuum until all liquid goes through the cartridge, and then turn off the vacuum source.

4. **Cartridge Wash**: Add 700 μL of **Wash Buffer** (containing ethanol) to the cartridge. Apply vacuum until all liquid goes through the cartridge and then turn off the vacuum. Remove the cartridge from the manifold and place into a 2 mL wash tube. Centrifuge at ≤12,000 x *g* for 1 min to remove all residual wash buffer.

5. **DNA Elution**: Place the cartridge into a 1.5 mL recovery tube. Add 50 μL of <u>warm</u> **TE Buffer** directly to the center of the cartridge. Incubate at room temperature for 1 min, then centrifuge at ≤12,000 x *g* for 2 min.

NOTE: Room temperature TE Buffer may be used for elution, but optimal recovery is obtained with TE warmed to 65 to 70°C.

Troubleshooting Guide

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

Technical Support

For further technical assistance please contact us at
(888) 267-4436 or by email at techsupport@origene.com. Technical support and troubleshooting guides for these products can also be found on our website at www.origene.com.

Related Products
To see our full line of PowerPrep™ purification products visit our website at www.origene.com.