Empirical bioscience

Observe the Difference

TRUST THE OUTCOME

EB Pure PCR Purification Kit

SS-EB-PPK-5: 5 Preps

EB-PPK-50: 50 Preps

EB-PPK-200: 200 preps

Made in Michigan

www.Empiricalbioscience.com

REGISTERED ISO 13485

EB Pure PCR Purification Kit Lab Manual

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Introduction

The EB Pure PCR Purification Kit is a convenient system for the fast and reliable purification of PCR products. The EB Pure PCR Purification Kit uses the High-Bind DNA Mini Column to recover DNA bands from 100 bp to 10 kb free of oligonucleotides, nucleotides, and polymerase with yields exceeding 80%. The binding conditions of the High-Bind DNA Mini Columns are adjusted by the addition of a specially formulated buffer before adding the sample. Following a rapid wash step, DNA is eluted with deionized water or a low salt buffer. Purified DNA can be directly used for most downstream applications include T-A ligations, PCR sequencing, restriction enzyme digestion, or various labeling reactions.

Benefits of the EB Pure PCR Purification Kit

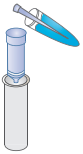
- **Fast -** DNA recovery from enzymatic reactions in less than 10 minutes
- **Reliability -** Optimized buffers that guarantee pure DNA
- **Safety -** No organic extractions
- **Quality -** Purified DNA is suitable for any application

Binding Capacity

Each High-Bind DNA Mini Column can bind ~30 µg DNA.

Centrifugation Protocol

Add CP Buffer



Transfer Lysate and Bind

Wash

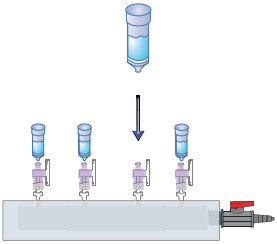
Dry

Elute

Vacuum Protocol

Add CP Buffer

Transfer Lysate and Bind



Wash

Dry

Elute

Kit Contents

Product Number	SS-EB-PPK-5	EB-PPK-50	EB-PPK-200
Preparations	5	50	200
High-Bind DNA Mini Columns	5	50	200
Collection Tubes (2 mL)	5	50	200
CP Buffer	5 mL	40 mL	150 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
Elution Buffer	5 mL	10 mL	20 mL

Storage and Stability

All of the EB Pure PCR Purification Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in CP Buffer. Dissolve such deposits by warm-ing the solution at 37°C and gently shaking.

Preparing Reagents

Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
SS-EB-PPK-5	6 mL
EB-PPK-50	60 mL
EB-PPK-200	100 mL per bottle

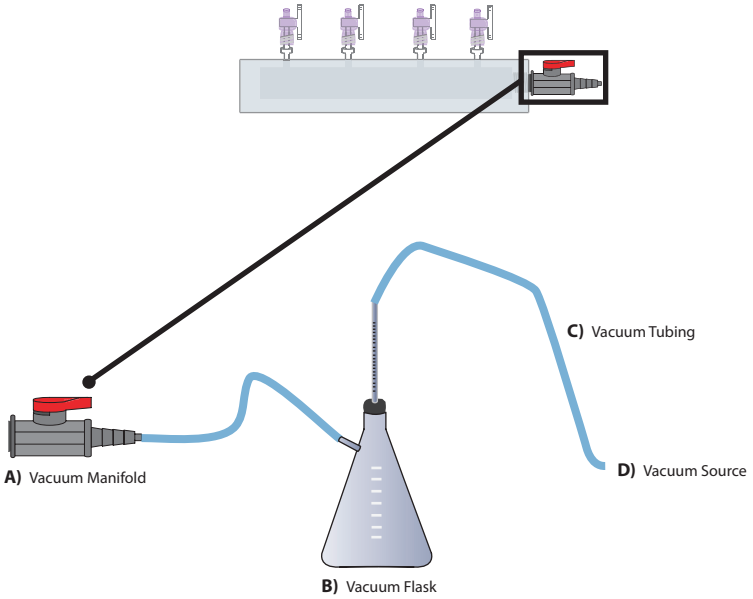
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold
Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



EB Pure PCR Purification Kit - Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: Sterile deionized water or TE Buffer
- For fragments <200 bp, 100% isopropanol

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube (not provided).

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.
   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and is smaller than 200 bp, you would use 500 µL CP Buffer and 40 µL 100% isopropanol.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Insert a High-Bind DNA Mini Column into a Collection Tube (2 mL) (provided).

7. Add the sample from Step 5 to the High-Bind DNA Mini Column.

8. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Add 700 µL DNA Wash Buffer.
    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse collection tube.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Centrifuge the empty High-Bind DNA Mini Column at maximum speed for 2 minutes to dry the column.
    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

15. Transfer the High-Bind DNA Mini Columns into a clean 1.5 mL microcentrifuge tube (not provided).

16. Add 30-50 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute.
    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

19. Store DNA at -20°C.

EB Pure PCR Purification Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: Sterile deionized water or TE Buffer
- For fragments <200 bp, 100% isopropanol

Before Starting:

- Prepare DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

2. Determine the volume of your PCR reaction.

3. Transfer the sample into a clean 1.5 mL microcentrifuge tube (not provided).

4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.
   Note: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100 µL and is smaller than 200 bp, you would use 500 µL CP Buffer and 40 µL 100% isopropanol.

5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.

6. Prepare the vacuum manifold according to manufacturer's instructions and connect the High-Bind DNA Mini Column to the manifold.

7. Transfer the entire sample to the High-Bind DNA Mini Column.

8. Switch on vacuum source to draw the sample through the column.

9. Turn off the vacuum.

10. Add 700 µL DNA Wash Buffer.
    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the Preparing Reagents section on Page 4 for instructions.

11. Switch on vacuum source to draw the DNA Wash Buffer through the column.

12. Turn off the vacuum.

13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.

14. Transfer the High-Bind DNA Mini Column into a Collection Tube (2 mL) (provided).

15. Centrifuge the empty High-Bind DNA Mini Column at maximum speed for 2 minutes to dry the column.
    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

16. Transfer the High-Bind DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

17. Add 30-50 µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.

18. Let sit at room temperature for 2 minutes.

19. Centrifuge at maximum speed for 1 minute.
    Note: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

20. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise.

Possible Problems and Suggestions

Low DNA Yields	
Not enough CP Buffer added to sample	Add more CP Buffer as indicated. For DNA fragments <200 bp in size, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol.
Water pH is too low (< 7.5)	Check the pH of the water, adjust the pH of the water to 8.0 using Tris-HCl (2M, pH 8.5).
No DNA eluted	
DNA Wash Buffer was not diluted with 100% ethanol	Prepare DNA Wash Buffer as instructed on the bottle, or refer to Page 4.
Optical densities do not agree with DNA yield on agarose gel	
Trace contaminants eluted from column will increase A_{260}	Make sure to wash column as instructed in Steps 10-13 of either protocol. Rely on agarose gel/ethidium bromide electrophoresis for quantification.
DNA sample floats out of well while loading agarose gel	
Ethanol not completely removed from column	Centrifuge as instructed in Step 14 of the centrifugation protocol and Step 15 of the vacuum protocol to completely dry the column matrix.